Enzymatic kinetic resolutions combined with racemisation techniques: powerful methodology for asymmetric synthesis

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Enzymatic *Kinetic Resolutions* Combined With Racemisation Techniques: Powerful Methodology for Asymmetric Synthesis.

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of the University of Bath
1998

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Dedication.

Dedicated to my parents for
continuing help and
support.

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I would like to take this opportunity to thank my supervisor, Prof. Jon Williams for his guidance, support, and enthusiasm throughout this work. Without his guidance things could have gone horribly wrong.

I would also like to thank the support staff at Loughborough University, Alistair Daley and John Kershaw. Many thanks also to the support staff at Bath University, John Bradley and Russell Barlow for sorting out the chemical orders on time.

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Thanks to the organic chemistry researchers who have been great fun, and to their bosses.

Finally I am grateful to EPSRC for providing the money.

Finally thanks to my parents for being so nice and supportive throughout my years of scrounging money off them.

Abbreviations.

aq.	aqueous
Asp.	aspartine
iBu	isobutyl
tBu	tertbutyl
DMAP	4-dimethylaminopyridine
DCM	dichloromethane
de	diastereomeric excess
ee	enantiomeric excess
EtOAc	ethyl acetate
$Eu(hfc)_3$	tris[3(heptafluoropropyl) hydroxymethylenecamphorato] europium (III).
GC	gas chromatography
His.	histidine
hrs.	hours
HPLC	high performance liquid chromatography
i	iso
IR	infra-red
mins.	minutes
NMR	nuclear magnetic resonance
p	para
Ph	phenyl
iPr	isopropyl
prep. TLC	preparative thin layer chromatography
sat.	saturated
Ser.	serine
t	tert
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	trimethylsilyl
pTSA	paratoluenesulfonic acid
wrt	with respect to

Notes.

Conversion is in respect to the amount of product produced by the reaction.

Abstract.

Enzymes have found many applications in modern synthetic organic chemistry, particularly in the *kinetic* hydrolysis of esters and amides. Do to the nature of an enzymatic *kinetic resolution* the desired product can never be isolated in yields greater than 50 % without the loss of enantioselectivity.

In order to overcome this inherent drawback, the enzymatic *kinetic resolution* was combined with an *in situ* racemisation protocol to afford a *dynamic resolution* allowing one enantiomer of product to be isolated in yields over 50 %.

The above technique was applied to the *dynamic resolution* of oxa-acetates, allylic acetates, thioacetates and α-bromo esters.

Table of Contents.

1.0 Introduction. 3

1.1 Introduction. 4
1.2 Enzymes and Organic Synthesis. 4
1.3 Hydrolytic enzymes. 4
1.4 Mechanism of Enzymatic Hydrolysis. 5
1.5 Lipases and *Kinetic Resolutions*. 6
1.6 *Dynamic Resolutions*. 8

2.0 Studies Towards the *Dynamic Resolution* of allylic acetates. 13

2.1 Introduction. 14
2.2 Preparation of Allylic Acetates. 22
2.3 Enzymatic *Kinetic Resolution* of Allylic Acetates. 25
2.4 Palladium Catalysed Racemisation of Allylic Acetates. 30
2.5 Racemisation of 4-Acetoxy Penten-2-ene. 32
2.6 Conclusions. 33

3.0 Studies Towards the *Dynamic Resolution* of Non-Allylic Acetates. 34

3.1 Introduction. 35
3.2 Preparation of Acetates. 46
3.3 Enzymatic *Kinetic Resolution* of Acetates. 47
3.4 Racemisation of 1-Phenethyl Acetate. 50
3.5 Synthesis of Acetoxy Methyl Mandelate. 54
3.6 Enzymatic *Kinetic Resolution* of Acetoxy Methyl Mandelate. 54
3.7 Racemisation of Acetoxy Methyl Mandelate. 56
3.8 *Dynamic Resolution* of Acetoxy Methyl Mandelate. 60
3.9 Conclusions. 62

4.0 Studies Towards the *Dynamic Reolution* of Thioacetates. 65

4.1 Introduction. 66
4.2 Preparation of Thioacetates. 75
4.3 Analysis of o-Methoxy Phenethyl Thioacetate. 80
4.4 Analysis of Phenethyl Thioacetate. 81
4.5 Enzymatic *Kinetic Resolution* of Thioacetates. 82
4.6 Racemisation of Phenethyl Thioacetate. 87
4.7 Conclusions. 90

5.0 Studies Towards the *Dynamic Resolution* of α-Bromo Esters. 94

5.1 Introduction. 95
5.2 Preparation of α-Bromo Esters. 104
5.3 Enzymatic *Kinetic Resolution* of α-Bromo Esters. 106
5.4 Cross-linked Enzyme Crystals, CLEC Enzymes. 111
5.5 Selective Racemisation of α-Bromo Esters. 113
5.6 *Dynamic Resolution* of Methyl-α-Bromo Phenyl Acetate. 118
5.7 Polymer Bound Phosphonium Bromide Salts. 121
5.8 Conclusions. 126

6.0 Experimental Section. 129

6.1 General Experimental. 130
6.2 Chapter 2 Experimental. 132
6.3 Chapter 3 Experimental. 141
6.4 Chapter 4 Experimental. 146
6.5 Chapter 5 Experimental. 162

7.0 List of Relevant Publications. 169

1.0 Introduction.

1.1 Introduction.

For many years enzymes have been part of day to day life. From the bread we eat to the soap powders with which we wash our clothes, all these contain or are manufactured using enzymes.

Enzymes are also part of our bodies. Thay catalyze many different and diverse functions within us, thus enzymes can be thought of as biological catalysts. All living systems use enzymes in the production of proteins, RNA, DNA, and other complex molecules. These help decompose foods into substances that we need for energy and to survive.

There are literally thousands of different enzymes and many preparations are mixtures of different enzymes, for example Bakers yeast which is used to help bread to rise is a complex mixture of different enzymes which can convert sugars into carbon dioxide and ethanol.

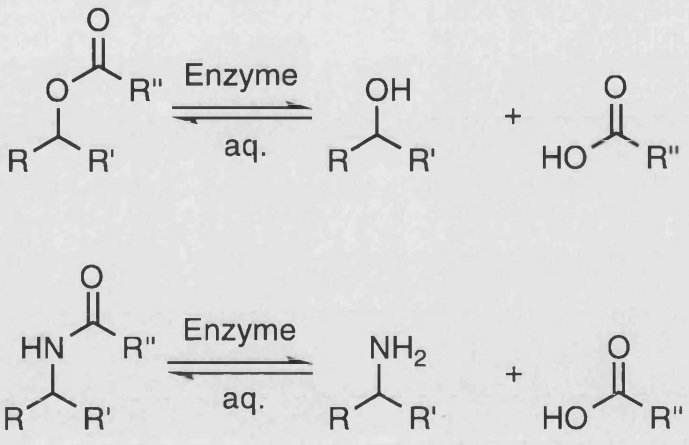
1.2 Enzymes and Organic Synthesis.

Enzymes have found practical application in organic synthesis, from Louis Pasteur to the large-scale industrial asymmetric syntheses of Ephedrine. In more recent years enzymes have found their way into research laboratories and are now frequently used in small scale synthesis. [1]

Enzymes are capable of catalyzing many different organic reactions but have the additional advantage that they can carry out these transformations under very mild conditions, often at neutral pH and ambient temperatures.

1.3 Hydrolytic Enzymes.

Hydrolytic enzymes are a family of enzymes that catalyze the hydrolysis of organic molecules, the most important being esters and amides, **Scheme 1**.



Scheme 1.

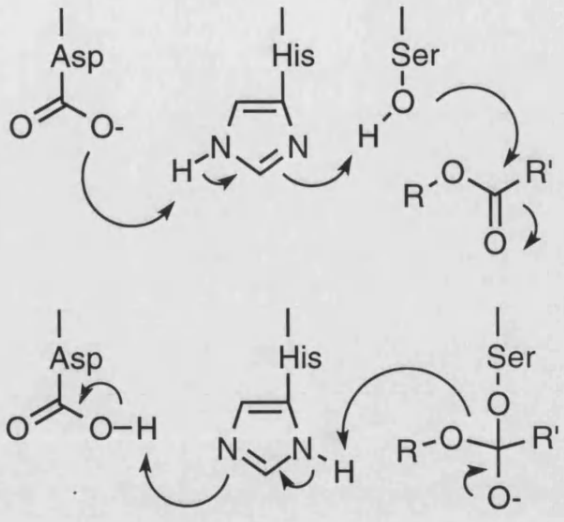
In the presence of excess water, hydrolytic enzymes hydrolyze esters to alcohols and carboxylic acids, amides to amines and carboxylic acids. But if we use an organic solvent then these reactions can be reversed, i.e. esters can be formed from an acid and an alcohol.

There are four differing groups of hydrolytic enzymes, lipases, esterases, amidases and proteases with different enzymes of the same family displaying different selectivity, chemical activity and other properties. These features can be utilized to perform many different transformations.

1.4 Mechanism of Enzymatic Hydrolysis.

Hydrolytic enzymes operate via many different mechanisms, some of which are understood.

Pseudomonas sp. lipase is an example of the serine protease enzymes which use an aspartine, histidine, and serine group to hydrolyze esters. These three amino acid residues are all attached to the same protein and it is the way in which the protein folds and brings these three amino acids together that forms the active site of the lipase. The ester function is then captured in the active site and hydrolysis takes place by the activated OH nucleophile on the serine function, **Scheme 2**.

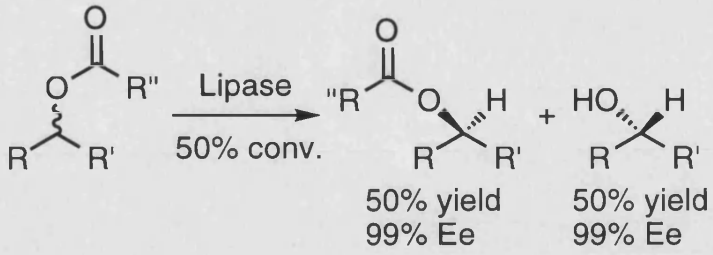


Scheme 2.

During the reaction carboxylic acid is produced, therefore enzymatic ester hydrolyses are usually carried out in a buffered solution to maintain the pH of the reaction as close to pH 7.0 as possible. Varying the pH of an enzymatic reaction may alter the conformation and / or the ionization status of the active site and alter the substrate specificity which, in turn, can lower the enzyme's ability to function.

1.5 Lipases and *Kinetic Resolutions.*

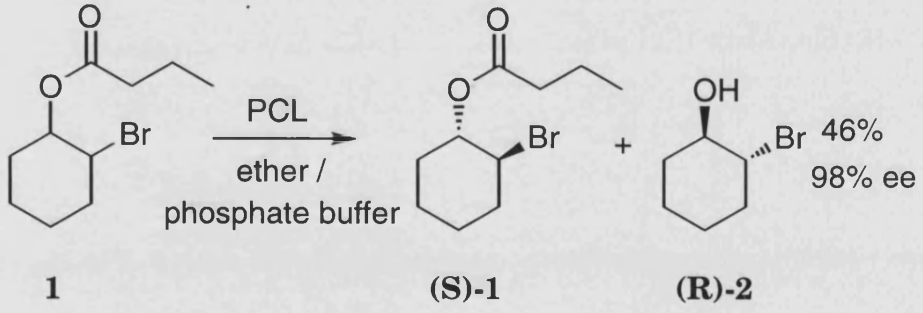
Due to the fact that the active sites of these enzymes are formed from amino acids they contain an asymmetric environment which results in reaction of only one enantiomer of the substrate. This can be utilized to provide esters or alcohols with very high enantioselectivity. If a racemic mixture of an ester is treated with a lipase in an aqueous solution then one enantiomer of the original ester will be hydrolyzed to the corresponding alcohol while the other enantiomer will remain un-hydrolysed and will remain as ester, **Scheme 3**.



Scheme 3.

It could be said that the R-enantiomer reacts faster than the S-enantiomer, i.e. $k_R > k_S$ and we have resolved these two enantiomers. We have a *kinetic resolution*. For an ideal resolution one enantiomer reacts much faster than the other, i.e. $k_R >> k_S$ and at 50% conversion a mixture of 50% (R)-enantiomer and 50% (S)-enantiomer is present, both of which are > 99% enantiomeric excess (ee).

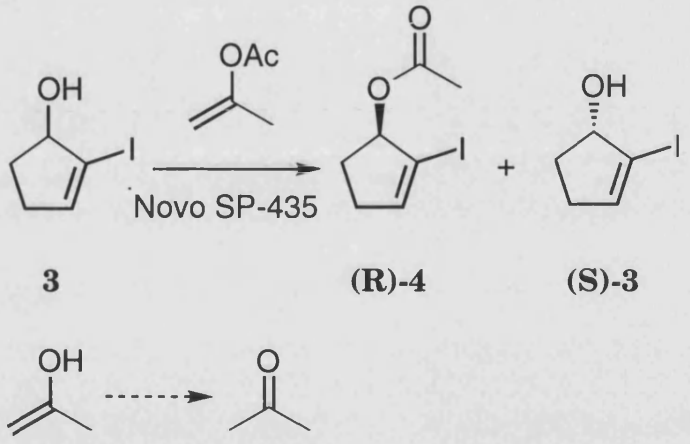
A good example of the *kinetic resolution* of a cyclic ester using a lipase was demonstrated by Gupta and Kazlauskas ,[2] where he treated a bromo-cyclohexyl butyrate **1** with *Pseudomonas cepacia* lipase (PCL) in an ether / phosphate buffer system to form the alcohol **2** which was resolved in good yield and enantiomeric excess, **Scheme 4**.



Scheme 4.

As stated earlier hydrolytic enzymes are also able to catalyse the reverse reaction, i.e. the esterification of alcohols. This can be achieved by carrying out the reaction in an organic solution instead of an aqueous system [3], **Scheme 5**. This is an example of an enzymatic esterification, which is potentially reversible, but if the aryl donor is carefully selected then the enzyme will be unable to perform the

reverse hydrolysis. In the example given isopropenyl acetate is used as the aryl donor to esterify the iodo cyclopentenol **3** to the cyclic acetate **4**. Once the iodo-alcohol **3** is esterified the resulting vinylic alcohol is subject to keto-enol tautomerism and is then unable to participate in the reverse reaction .



Scheme 5.

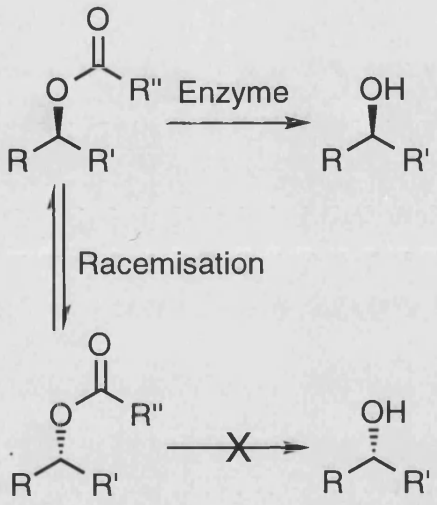
1.6 *Dynamic Resolutions.*

Because of the nature of a *kinetic resolution* the overall yield of the desired product can never be over 50% without the loss of enantiomeric excess. The unwanted enantiomerically enriched starting material is either discarded or 'recycled' by further chemical manipulation. This can be both costly and time consuming. One way to overcome this inherent waste of chiral material is to perform a *dynamic resolution.*

A *dynamic resolution* can take place when the two enantiomers of starting material are in a dynamic equilibrium. The enzyme will only react with one enantiomer and therefore if the starting material is continually being racemised then there will always be a supply of that particular enantiomer in the reaction until all starting material has been consumed and converted into chiral product, **Scheme 6**.

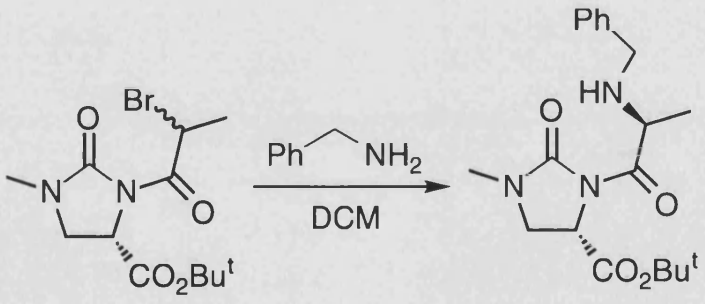
In order for this methodology to succeed the product of the enzymatic conversion must not undergo racemisation by the racemisation conditions. For example in the case of an ester hydrolysis if the

product alcohol is racemised along with the starting ester then the high selectivity of the enzyme is wasted as racemic alcohol will be produced at the end of the reaction.



Scheme 6.

There is a literature precedent for chemically mediated *dynamic resolutions*, Nunami *et al* [4] displayed the application of *tert*-butyl (4S)-1-methyl-2-oxoimidazolidin-4-carboxylate as an effective chiral auxiliary in a S_N2 displacement of bromine by benzylamine under base-catalyzed racemisation, **Scheme 7**.



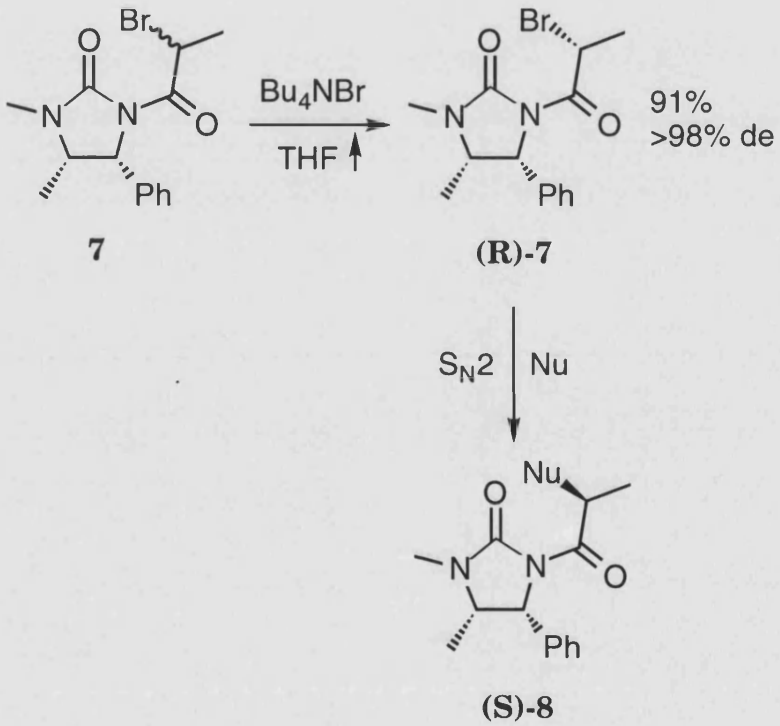
5 **6**

Scheme 7.

In this example the substrate **5** is racemised by deprotonation of the substrate followed by the subsequent attack of the nucleophile to

displace the bromine to afford **6**. Nunami [5] and Caddick [6] have both proposed mechanisms to explain the stereochemistry of the reaction.

Another interesting *dynamic resolution* utilizing a similar chiral auxiliary has been demonstrated by Caddick and Jenkins [7], **Scheme 8**. In this example a bromide source was used to epimerise the substrate **7** while allowing the solvent to evaporate. This resulted in the less soluble 2-(R)-enantiomer to be deposited which could then be isolated and treated with an amine nucleophile to produce the chiral (S)-imino acid derivative **8**.



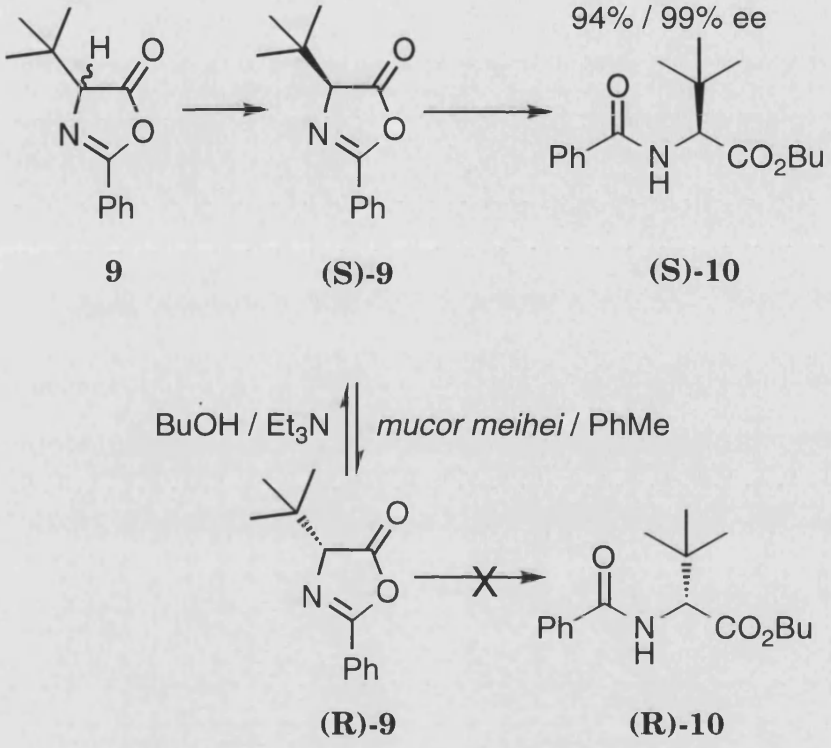
7 **(R)-7** **(S)-8**

Scheme 8.

There are relatively few examples of enzyme mediated *dynamic resolutions* in the literature but one interesting example was developed by Turner and Winterman [8] towards the *dynamic resolution* of oxazolinones, **Scheme 9**.

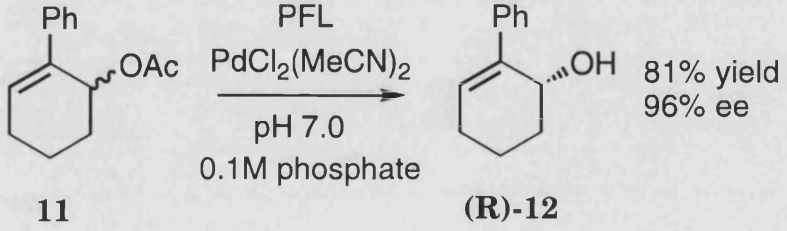
Here the α-proton is made more acidic by the iminyl and carbonyl functions, thus deprotonation results in racemisation of the substrate **9**. If this oxazolinone is treated with butanol in the presence of immobilized *mucor miehei* lipase, then a transesterification takes

place to yield the butyl ester **10** in good yield and enantiomeric excess. This butyl ester can easily be converted into the natural form of *tert*-leucine.



Scheme 9.

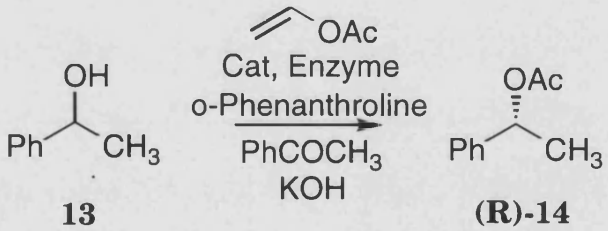
Another interesting example is the combination of an enzymatic *kinetic resolution* with a transition metal catalyzed racemisation devised by Williams and Allen [9] from our own group, **Scheme 10**.



Scheme 10.

In this example the cyclohexenyl acetate **11** is racemised *in situ* using 5 mol% $PdCl_2(MeCN)_2$ to catalyse a [3,3]-sigmatropic acetate rearrangement. Hydrolysis was carried out by treatment with *Pseudomonas florescence* lipase (PFL) in a 0.1M phosphate buffer, to yield the enriched allylic alcohol **12** in excellent yield and enantiomeric excess.

In a later paper Williams *et al* [9b] demonstrated the *dynamic resolution* of phenethyl alcohol **13** to the corresponding acetate **14**, **Scheme 11**.



13 **(R)-14**

Cat.	Enzyme	Time hrs	Temp °C	Conv. %	(OAc) ee %
$[Ir(coe)Cl]_2$	PSL	96	60	91	2
$Al(Oi\text{-}Pr)_3$	PFL	72	80	86	20
$[Rh(cod)Cl]_2$	PFL	144	50	76	80
$Rh_2(OAc)_4$	PFL	72	20	60	98

Scheme 11.

In this example the alcohol **13** is racemised via a metal catalysed transfer hydrogenation mechanism and when this process is combined with an enzymatic acetylation using *Pseudomonas fluorescens* Lipase (PFL) the (R)-acetate **14** can be isolated in very good enantiomeric excess and conversion.

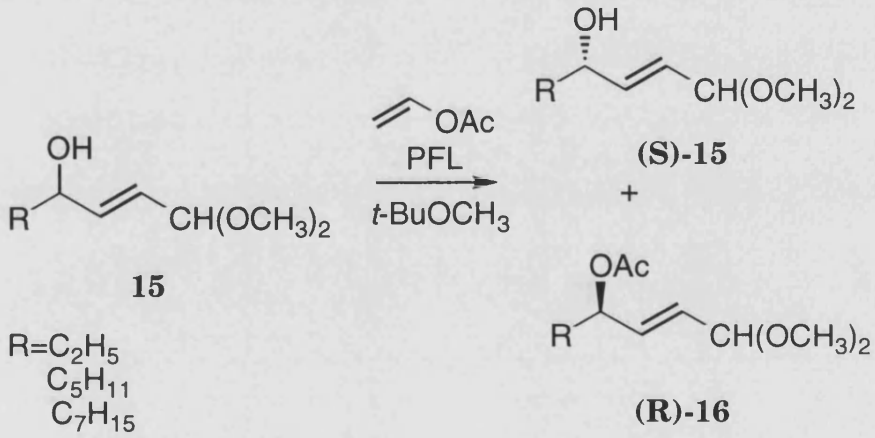
It was our aim to extend a racemisation / enzyme methodology further and design *dynamic resolutions* using enzymatic hydrolysis combined with a racemisation technique in order to produce enantiomerically pure compounds in high yields.

2.0 Studies Towards the *Dynamic Resolution* of Allylic Acetates.

2.1 Introduction.

As stated in Chapter 1 enzymes are finding strong application in modern synthetic organic chemistry due to the high level of stereoselectivity displayed by these catalysts, [10,11] and many are directed towards the synthesis of allylic products.

Allevi *et al* [12] conducted a study into the enzymatic esterification of various hydroxy-acetals with a view to study further the biological properties of the hydroxy-acetals, **Scheme 12**.

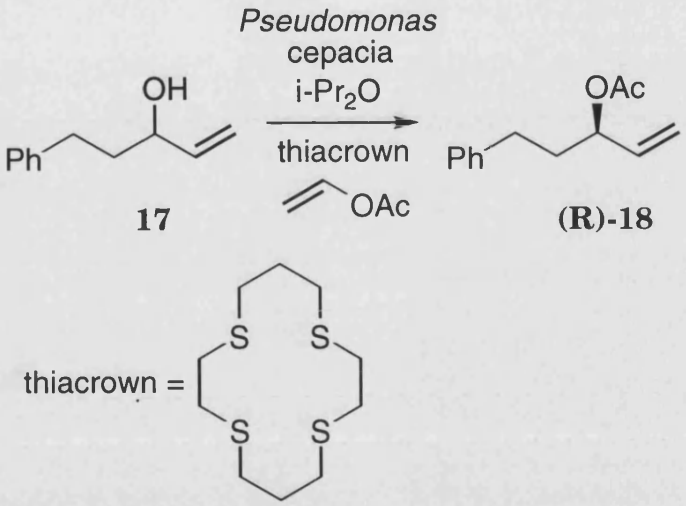


R	Conv. %	(S)-OH ee %	(R)-OAc ee %
C_2H_5	51	> 95	90
C_5H_{11}	52	> 95	89
C_7H_{15}	52	> 95	88

Scheme 12.

In this example the hydroxy acetals were treated with PFL in vinyl acetate and *tert*-butyl methyl ether and yielded both acetate and alcohol in very good enantiomeric excess and yield. The results show that the selectivity of the enzyme is not affected by the length of the R substituent in the enzymatic *kinetic resolution*.

Takagi *et al* recently published an interesting paper highlighting the enantioselectivity and reaction rate enhancement of a PCL catalysed acetylation of 5-phenyl-1-penten-3-ol, [13] **Scheme 13**.



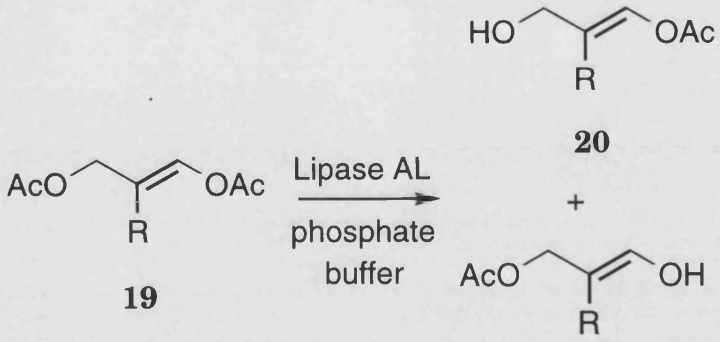
Additive	Solvent	Time h	(R)-OAc ee %	Conv. %
none	hexane	93	97	38
thiacrown	hexane	93	97	50
none	i-Pr$_2$O	65	98	48
thiacrown	i-Pr$_2$O	65	> 99	49

Scheme 13.

These reactions were carried out using no more than 0.5 mol % of the thiacrown (wrt substrate) and the authors postulate that the thiacrown will bind the allylic alcohol and facilitate the penetration of the allylic alcohol into the enzymes active site, though the validity of this arguement is debatable.

Hydrolysis of allylic acetates are also catalysed by hydrolytic enzymes [14]. Itoh *et al* [15] demonstrated the regioselectivity demonstrated by Lipase AL (*Achromobacter sp.*) in the hydrolysis of a diacetate, **Scheme 14**.

Perfect regioselectivity was observed when R=Bu, or R=n-Hept were subjected to the reaction conditions.

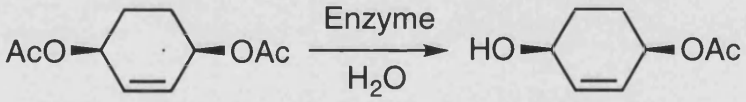


R=CH_3, Et, Bu, n-Heptyl

R	time hr	regioselectivity	yield %
		20 : 21	**20**
CH_3	1.5	88 : 12	50
Et	1	100 : 0	65
Bu	120	100 : 0	50
n-Heptyl	120	100 : 0	85

Scheme 14.

In a similar reaction Harris *et al* [16] reported the synthesis of (3S, 6R)-3-hydroxy-6-acetoxy-cyclohex-1-ene from the diacetate substrate cis-3, 6-diacetoxycyclohex-1-ene, **Scheme 15**.



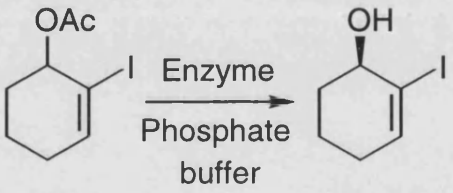
22 **(S,R)-23**

Enzyme	time hr	yield %	ee %
Aspergilus niger sp.	2	27	0
Chromobacterium sp.	2	51	47
Pig liver esterase (PLE)	7	59	49
Pseudomonas sp. (PCL)	6	64	79

Scheme 15.

In this example the lipase from *Pseudomonas cepacia* (PCL) was found to be the most successful, yielding the mono-acetate in 64% yield with an enantiomeric excess of 79%.

In the study of the *Candida antartica* lipase catalysed transesterification of 2-iodo cycloalkenols, Johnson and Sakaguchi [17] also studied the hydrolysis of cyclic iodo acetate using Amano PS-30 and Novo SP-435 lipases, **Scheme 16.**



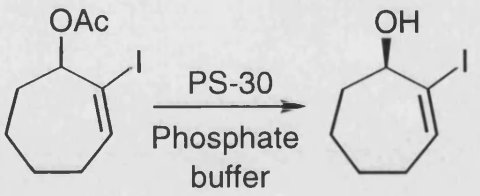
24 **(R)-24**

Enzyme	time hr	Conv. %	(R)-OH ee %	(S)-OAc ee %
PS-30	0.5	60	80	99.9
SP-435	18	52	87	> 95

Scheme 16.

In the above examples both PS-30 and SP-435 produce the (R)-enantiomer in good enantiomeric excess though conversion had risen above 50%. It is also noteworthy that the enantiomeric excess of the remaining acetate is a calculated value and not a measurement.

The authors also demonstrated that PS-30 can tolerate larger ring molecules, **Scheme 17**. Here an acetate is hydrolysed to the corresponding alcohol on a seven membered ring system in good enantiomeric excess.

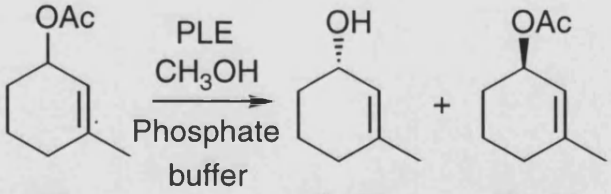


26 **(R)-27**

Enzyme	time hr	Conv. %	(R)-OH ee %	(S)-OAc ee %
PS-30	5	60	73	99.9

Scheme 17.

In a recent publication investigating the enzyme mediated synthesis of Seudenol and 1-methyl-2-cyclohexen-1-ol, the aggregation pheromone of *Dendroctonus pseudotsugae* , [18] Mori and Ogoche studied the enzymatic resolution of Seudenol acetate by pig liver esterase (PLE), **Scheme 18**.

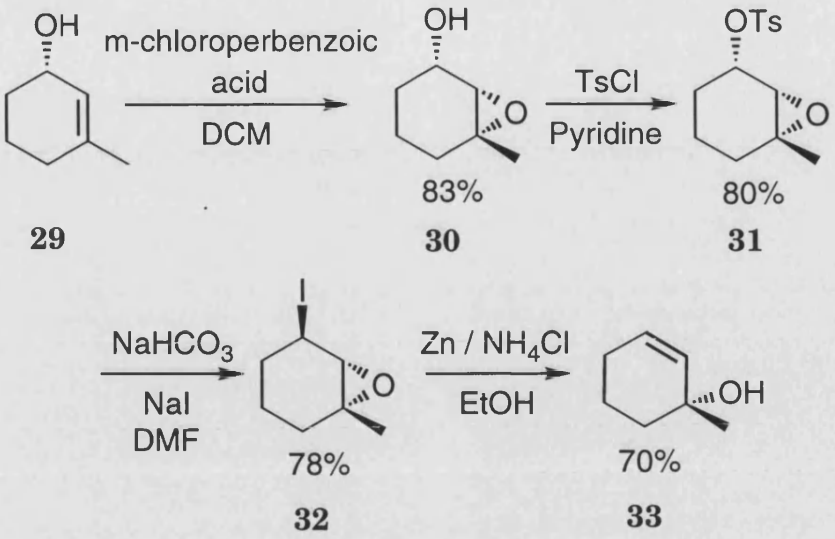


28 **(S)-29** **(R)-28**

Scheme 18.

After the seudenol acetate was incubated with PLE in a 0.1M phosphate buffer and methanol solution for 72 hrs at 10 °C a 90% yield of the partially resolved seudenol was obtained. Re-acetylation of this seudenol followed by further reaction with PLE to 75% conversion yielded (S)-seudenol $[\alpha]^{23} = -60$ ($CHCl_3$) in good yield.

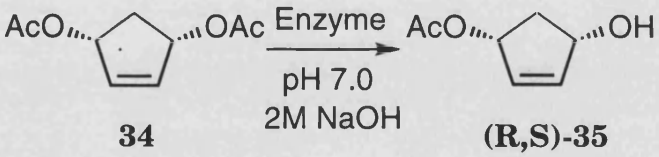
The authors then use the enantiomerically enriched (S)-seudenol in the gram scale synthesis of the pheromone in 70% yield, **Scheme 19.**



Scheme 19.

(S)-Seudenol was epoxidised using m-chlorobenzoic acid in good yield, then the alcohol function was tosylated using tosyl chloride in pyridine to yield the tosylated product **31** which was recrystallised from pentane. The tosylate **31** was treated with sodium iodide and sodium hydrogen carbonate in DMF to yield the iodide **32** in 78% yield. Reductive elimination of the iodide using zinc powder and a catalytic amount of ammonium chloride yielded the pheromone (R)-1-methyl-2-cyclohexen-1-ol **33** in 70% yield.

This is an interesting example of the use of an enzymatic resolution in the synthesis of an optically active natural product.



Lipase	Conv. %	Product	% yield	% ee
Porcine Pancreas	52	(1R, 4S)	87	92
Pseudomonas sp.	50	(1R, 4S)	80	92
Mucor miehei	50	(1R, 4S)	85	95
Chromobacterium viscosium	50	(1R, 4S)	76	91

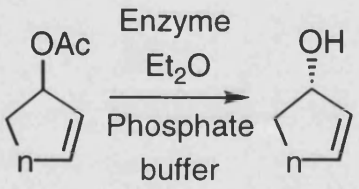
Scheme 20.

Laumen and Schneider [19] employed a regioselective enzymatic desymmetrisation in the preparation of enantiomerically pure cyclopentanoid chiral building blocks, **Scheme 20**.

Here we see that all four enzymes highlighted above are capable of desymmetrising the cyclic diacetate in good yields and enantiomeric excesses. Laumen and Schneider used the allylic hydroxy acetates and further manipulated them to form chiral building blocks for cyclopentoid natural products.

In a more recent study Gupta and Kazlauskas [20] investigated the enzymatic hydrolysis of a series of cyclic alcohols **36**, **Scheme 21**.

It was revealed that the direct resolution of the allylic alcohols proceeded with very low enantioselectivity because the two substituents at the stereocentre (CH_2-CH_2 vs. CH=CH) were similar in size. To improve the selectivity of the enzymes the allylic alcohols were converted into 2-bromocycloalkanols **38**.

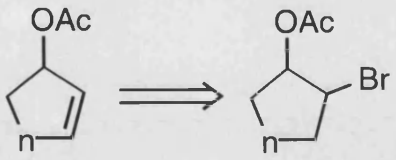


36 **(S)-37**

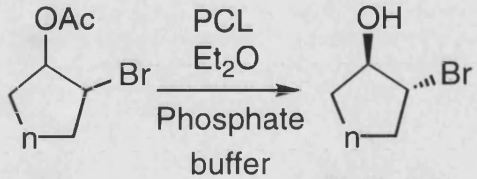
n	Enzyme	Conv. %	(OH) ee %
2	CE	47	8
2	PCL	46	5
2	CRL	44	9

Scheme 21.

These 2-bromocycloalkanols **38** were then screened with the enzymes, **Scheme 22** and the enantioselectivity of the enzymes increased dramatically.



36 **38**



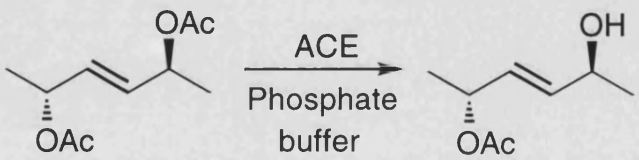
38 **(R,S)-39**

n	Enzyme	Conv. %	(OAc) ee %	(OH) ee %
1	CE	31	43	95
1	PCL	41	66	95
2	CE	43	75	98
2	PCL	51	98	94
3	CRL	40	67	98

Scheme 22.

As demonstrated here if the enzymatic *kinetic resolution* is unsatisfactory then manipulation of the substrate can result in better enantioselectivity.

Schink and Backvall [21] investigated the enzymatic hydrolysis of meso-2, 5-diacetoxy-3-hexene **40** to yield (2S, 5R)-5-acetoxy-3-hexen-2-ol **41** using *Acetylcholine* esterase (ACE), **Scheme 23**.



40 **(S,R)-41**

Scheme 23.

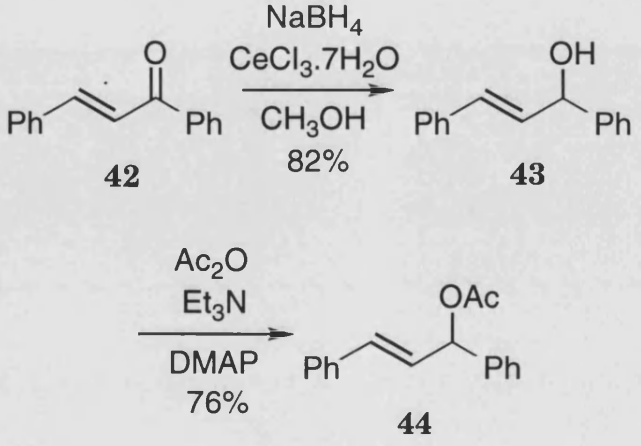
Here the diacetate was desymmeterised in 77% yield and 92% enantiomeric excess using ACE and a 0.1M phosphate buffer system.

The product monoacetate was then manipulated further to synthesise both enantiomers of a bee pheremone.

In all the examples of *kinetic resolutions* given above the unwanted side product is either discarded, manipulated further chemically or recycled and subjected to the *kinetic resolution* again. This is either wasteful or time consuming so we aimed to apply the chemistry of Allen and Williams (Chapter 1) to a series of non-cyclic allylic acetates to perform a *dynamic resolution* and thus remove the need for recycling unwanted enantiomers of optically active material.

2.2 Preparation of allylic acetates.

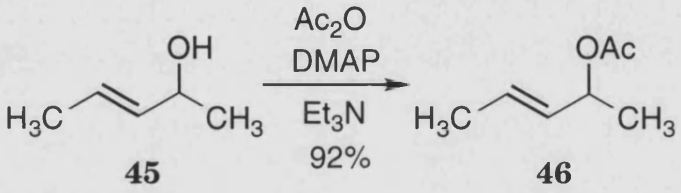
The allylic acetate. **44** was initially synthesised due to the ready availability of the starting materials from within the group, **Scheme 24**. The substrate was easily prepared in two steps from chalcone **42**. The regioselective 1, 2-reduction of chalcone **42** using Luche conditions , [22] cerium (III) chloride heptahydrate and slow addition of sodium borohydride at 0 °C. Upon purification by flash chromatography, analysis of the product by infra-red spectroscopy revealed the loss of the C=O band at ~ 1700 cm^{-1} and the appearance of the OH band at 3017 cm^{-1}.



Scheme 24.

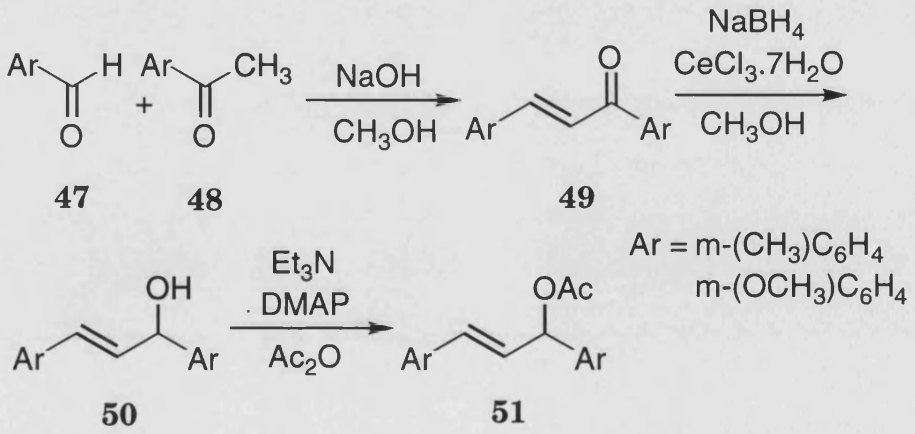
1,3-Diphenyl propen-1-ol **43** was acetylated with acetic anhydride and a catalytic amount of DMAP in triethylamine, following an aqueous work up 1,3-diphenyl propenyl acetate **44** was isolated in good yield. Characterisation of the product by NMR spectroscopy provided comparable results to the literature values. [23]

4-Acetoxy pent-2-ene **46** was similarly prepared by the acetylation of 3-penten-2-ol **45** using acetic anhydride, DMAP, and triethylamine, **Scheme 25**. Following an aqueous work up and flash chromatography the allylic acetate **46** was isolated in excellent yield. Comparison of NMR data with literature values [24] provided a suitable characterisation of the product.



Scheme 25.

Synthesis of the meta-substituted aromatic allylic acetates was quite straightforward, **Scheme 26**.



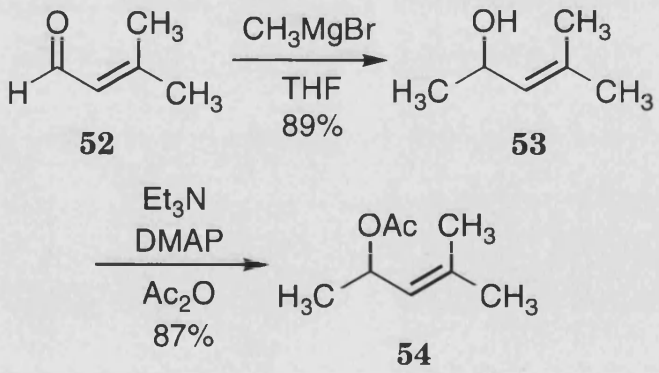
Scheme 26.

Formation of the α-β unsaturated ketones m-methyl-1,3-diphenyl propenyl acetate and m-methoxy-1,3-diphenyl propenyl acetate **51** was carried out under Claisen Schmidt condensation conditions, where an arylaldehyde **47** was reacted with the corresponding aryl methyl ketone **48** in a basic methanolic solution. [25] These reactions proceeded in good yield and formation of the α-β unsaturated ketones **49** is indicated by the protons in the 7.31-7.80 ppm (J=15.7-15.8 Hz) region of the ^{1}H NMR spectra of **49**. These signals are shifted down field by the high level of conjugation in these compounds.

Further proof can be found in the infra-red spectra of these compounds. A strong absorption at 1650-1670 cm^{-1} region represents the C=O $_{(str)}$ frequency of a carbonyl moiety. These rather low values are recorded due to the high amount of conjugation present in the molecules.

Reduction of the ketones **49** to the allylic alcohols **50** was accomplished by using Luche conditions and then acetylated using acetic anhydride, triethylamine, and a catalytic amount of DMAP to yield the allylic acetates **51** in very good yields.

4-Methyl-2-acetoxypent-3-ene **54** was easily synthesised from the commercially available 3-methyl-2-butenal **52** and CH_3MgBr, **Scheme 27**.



Scheme 27.

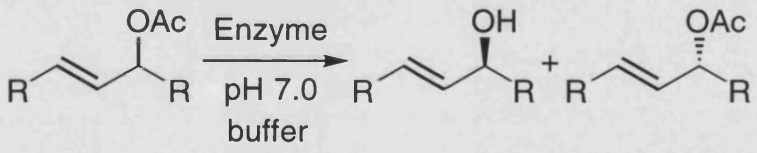
The α-β unsaturated aldehyde **52** is subjected to methyl Grignard attack to produce the α-β unsaturated alcohol 4-methyl penten-2-ol

53 in very good yield. This is then easily acetylated using familiar triethylamine, acetic anhydride, and DMAP conditions. Following flash chromatography 4-methyl-2-acetoxypent-3-ene **54** was isolated in an 87% yield.

2.3 Enzymatic *Kinetic Resolution* of allylic acetates.

With our series of allylic acetates in hand it was necessary to find optimum conditions for a *kinetic resolution* for each substrate. We chose to use enzyme preparations from the major commercial sources.

The general reaction scheme for the enzymatic *kinetic resolution* is shown, **Scheme 28** but for various reasons each substrate was screened under slightly different conditions.

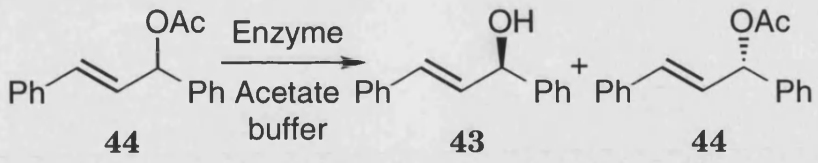


Scheme 28.

The substrate in an aqueous buffer (pH 7.0) was treated with the enzyme and the pH followed throughout the reaction. When the pH fell to below pH 7.0. 0.1M NaOH was added via a syringe to compensate for the production of acetic acid.

1,3-Diphenyl propenyl acetate **44** was screened against our bank of enzymes in an aqueous ammonium acetate buffer, **Scheme 29**.

The products of the reaction were extracted into diethyl ether and filtered through a plug of silica to remove the enzyme. If required the products were separated using flash chromatography.



Enzyme	Time	Conv.	(OH)[a,b]	(OAc)[a]
	days	%	ee % / Config.	ee%
CCl	6	42	4 / (S)	5
PFL	6	39	6 / (R)	9
R. Niveus	12	17	2 / (R)	3
M. Javanicus	12	13	0 / (R)	2
P. Roqueforte	12	19	0 / (R)	5

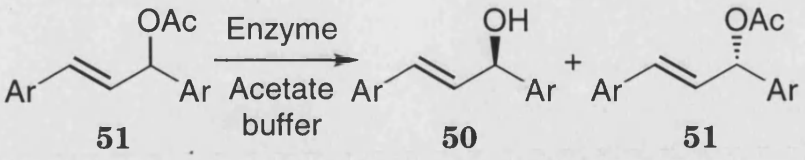
Reactions carried out at 38 °C. a) Enantiomeric excess was determined by HPLC analysis (Diacel Chiracel OD column, 99:1 n-hexane:IPA). b) Stereochemical configuration determined by comparison of HPLC spectra of alcohols of known stereochemistry.

Scheme 29.

As shown above none of the enzymes screened hydrolysed the allylic acetate **44** to the allylic alcohol **43** with good enantioselectivity, though *Candida cylidracea* lipase (CCL) and *Pseudomonas fluorescence* lipase (PCL) did process the starting material with a relatively good conversion. We decided to manipulate the starting material to increase the enantioselectivity without altering the substrate too much which could result in the loss of enzyme activity.

With the introduction of substituents on the terminal phenyl rings we again screened the allylic acetates **51** in an aqueous ammonium acetate buffer.

Reaction of m-methoxy-1,3-diphenyl propenyl acetate **51** (Ar= m-$(OCH_3)C_6H_4$) was carried out in an almost identical system to 1,3-diphenyl propenyl acetate **44**, **Scheme 30**.



Ar= m-$(OCH_3)C_6H_4$

Enzyme	Time	Conv.	(OH)[a]	(OAc)[a]
	days	%	ee %	ee%
PFL	6	37	19	24
CCL	4	32	1	6
PPL	6	26	1	3
PCL	4	29	8	14
M. Javanicus	12	22	3	2
R. Niveus	12	10	17	15

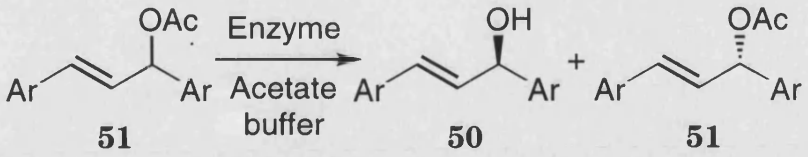
Reactions carried out at 38 °C. a) Enantiomeric excess determined by HPLC analysis.

Scheme 30.

Once again none of the enzymes screened in this study resolved the allylic acetate with a satisfactory enantiomeric excess. Though *Pseudomonas fluorescence* lipase (PFL) did produce allylic alcohol in almost 20% ee and was not improved upon.

Results of the enzymatic hydrolysis of m-methyl-1,3-diphenyl propenyl acetate **51** (Ar= m-$(CH_3)C_6H_4$), **Scheme 31** were similar. PFL displayed the best enantioselectivity but was still short of the theoretical maximum and so we decided further manipulation of the substrate was required.

It was decided that the phenyl termini may result in a poor 'fit' of the substrate into the enzyme active site. This could reduce the selectivity of the enzyme and so 4-acetoxy penten-2-ene **46** was synthesised and screened against our bank of enzymes.



51 **50** **51**

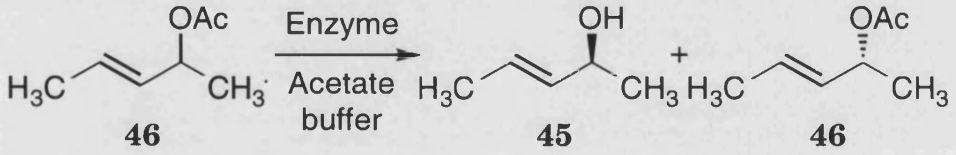
Ar= m-$(CH_3)C_6H_4$

Enzyme	Time days	Conv. %	(OH)[a] ee %	(OAc)[a] ee%
PFL	6	31	17	21
CCL	3	29	2	5
PCL	4	32	2	6
PPL	6	31	2	3

Reactions carried out at 38 °C. a) Enantiomeric excess determined by HPLC analysis.

Scheme 31.

As in the above examples the substrate was emulsified in an aqueous ammonium acetate buffer and then treated with the enzyme and the pH maintained at 7.0, **Scheme 32**.



46 **45** **46**

Enzyme	Time days	Conv. %	(OH)[a] ee %	(OAc)[a] ee%
PFL	2.5	48	99	98
PCL	2	70	37	49
PPL	3	17	16	21
CCL	2	20	31	30
R. Niveus	6	21	28	26
M. Javanicus	6	19	27	31

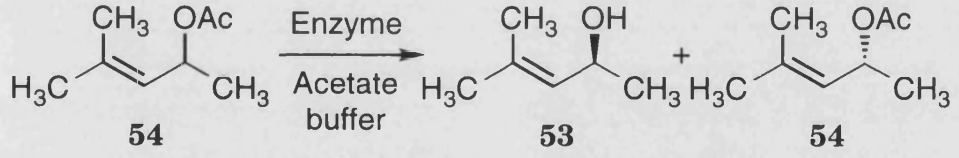
Reactions carried out at 40 °C. a) Enantiomeric excess determined by GC analysis (β-cyclodextrin, 25 m, 39 °C).

Scheme 32.

These results were encouraging. All enzymes screened displayed increased enantioselectivity, especially PFL which resolved the allylic acetate **46** in 99% enantiomeric excess at 48% conversion, this was an optimised result.

The rate of hydrolysis for this substrate was a lot quicker than the terminal phenyl substrates 1,3-diphenyl propenyl acetate **44**, m-methyl-1,3-diphenyl propenyl acetate **51** (Ar= $(CH_3)C_6H_4$), and m-methoxy-1,3-diphenyl propenyl acetate **51** (Ar= $(OCH_3)C_6H_4$). This could be due to the substrates ability to enter the active site of the enzyme without hindrance from large aryl groups.

We also studied the enzymatic *kinetic resolution* of 4-methyl-2-acetoxy-pent-3-ene **54**, **Scheme 33**. The substrate was once again screened against our bank of enzymes.



54 **53** **54**

Enzyme	Time days	Conv. %	(OH)[a] ee %	(OAc)[a] ee%
PFL	6	34	17	14
PCL	6	29	8	11
CAL	6	54	2	4
Allecaligens sp.	7	17	4	6
Geotrichium sp.	7	24	3	4

Reactions carried out at 40 °C. a) Enantiomeric excess determined by GC analysis (β-cyclodextrin, 25 m, 39 °C).

Scheme 33.

None of the enzymes screened provided a satisfactory resolution of 4-methyl-2-acetoxy pentene **54**. It is interesting to note that with the addition of the extra methyl group the enantioselectivity of the enzymes has fallen considerably, **Scheme 34**.

Substrate	Enzyme	Time	Temp	Conv.	(OH)[a]	(OAc)[a]
		days	°C	%	ee %	ee %
46	PFL	2	40	48	99	98
54	PFL	6	40	34	17	14
46	PCL	2	40	70	37	49
54	PCL	6	40	29	8	11

Reactions carried out at 40 °C. a) Enantiomeric excess determined by GC analysis (β-cyclodextrin, 25 m, 39 °C).

Scheme 34.

Here we can see that when treated with PFL 4-acetoxy penten-2-ene **46** was resolved to 48% conversion with 99% enantiomeric excess over 2 days. Compare with 4-methyl-2-acetoxy pentene **54**, where the PFL catalysed hydrolysis is considerably poorer, 34% conversion at 17% enantiomeric excess over 6 days. This trend is also mirrored in the PCL catalysed hydrolysis of the substrates.

Mention should also go to the rates of the reactions. The hydrolysis of **46** is considerably quicker than **54** with the same enzyme.

With the *kinetic resolution* of 4-acetoxy penten-2-ene **46** in hand we had to carry out investigations into a suitable racemisation methodology.

2.4 Palladium catalysed racemisation of allylic acetates.

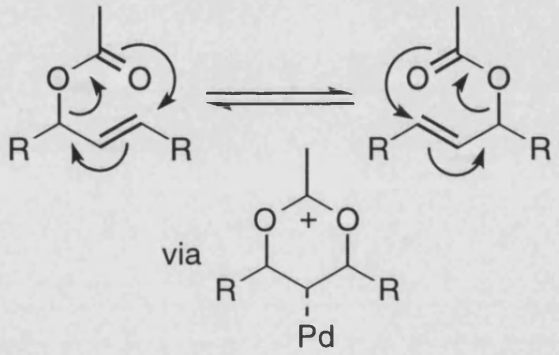
Allen and Williams from our own group achieved the *dynamic resolution* of a cyclic allylic acetate using an enzymatic *kinetic resolution* combined with a palladium (II) catalysed racemisation and we aimed to apply this methodology to 4-acetoxy pent-2-ene **46**.

In theory racemisation by palladium can be achieved by Pd(0) [26] and Pd(II) [27] complexes. Each different oxidation state has a different mechanism, both will be discussed below.

Palladium(II) racemisation of allylic acetates.

Palladium(II) complexes are able to catalyse [3, 3]-sigmatropic rearrangements, usually at low temperature and neutral pH.

The Pd(II) catalysed racemisation of an allylic acetate is shown below, **Scheme 35.** It is obvious that the corresponding alcohol will not be able to perform this rearrangement and therefore is not racemised and so will retain its chirality. This would make this technique applicable to our desired *dynamic resolution*.

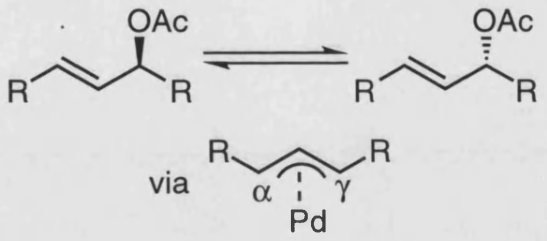


Scheme 35.

Palladium(0) racemisation of allylic acetates.

Palladium(0) species can effect a racemisation via the insertion of the Pd into the C-OAc bond thus forming an allylpalladium intermediate, **Scheme 36**.

Once formed the allylpalladium complex undergoes nucleophilic attack by another acetate at either the α or γ position to afford the two enantiomers of the original allylic acetate, i.e. a racemisation has taken place.

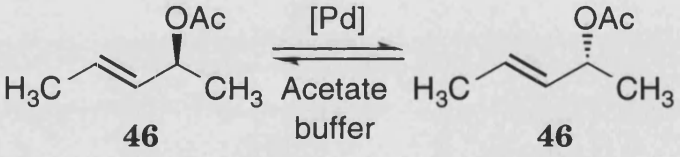


Scheme 36.

The acetate is eliminated from the opposite face to the co-ordinated palladium. Attack of the nucleophile, another acetate, also occurs at the opposite face to the palladium. This leads to an overall retention of configuration, but the position of nucleophilic attack is not necessarily at the same position of the original acetate hence racemisation takes place.

2.5 Racemisation of 4-acetoxy pent-2-ene 46.

Initial racemisation studies were focused on using palladium (0), **Scheme 37**. Here 10 mol% of palladium chloride allyl dimer was added to the chiral 4-acetoxy pent-2-ene (97% ee) **46** in ammonium acetate buffer. This was then stirred at room temperature over 18 hours. The reaction was then extracted with diethyl ether and filtered through silica but all starting material was consumed and yielded baseline material which was unidentified.



Scheme 37.

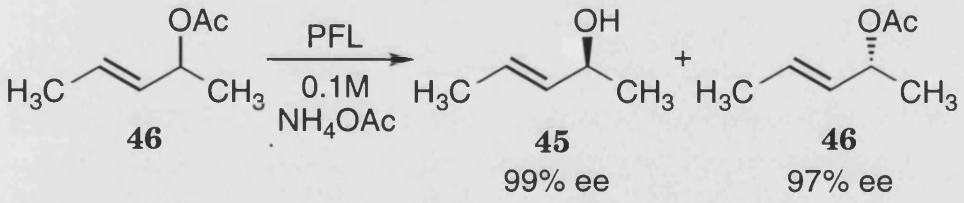
Next the methodology described by Allen and Williams was applied to our allylic acetate **46** Here 10 mol% of $PdCl_2(CH_3CN)_2$ was added to 1 mmol of resolved 4-acetoxy pent-2-ene (99% ee) **46** in 10 ml of ammonium acetate buffer and stirred at 38 °C overnight. Once again,

an organic extraction and filtration through silica yielded only polar base line material that was not identified.

2.6 Conclusions.

Here we have demonstrated the enzymatic *kinetic resolution* of 4-acetoxypenten-2-ene **46** using *Pseudomonas florescence* lipase (PFL) in 0.1M ammonium acetate buffer. The allylic alcohol **45** was isolated as one enantiomer (99% ee) at 48% conversion, and remaining acetate **46** in 97% ee, **Scheme 38**.

We also conducted some studies into the effect of substituent on lipase activity. A phenyl or m-substituted phenyl terminus was shown to hinder enzymatic activity, and enantioselectivity. But when replaced by a methyl group enzyme activity increased, resulting in shorter reaction times, and enantioselectivity was greatly improved. If a further methyl group is introduced, as in **54**, then once again we see the enantioselectivity of the enzyme fall along with an increase in the amount of time to reach a similar conversion.



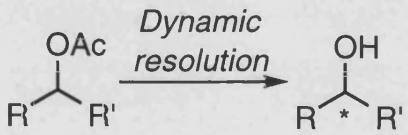
Scheme 38.

3.0 Studies Towards the *Dynamic Resolution* of Non-Allylic Acetates.

3.1 Introduction.

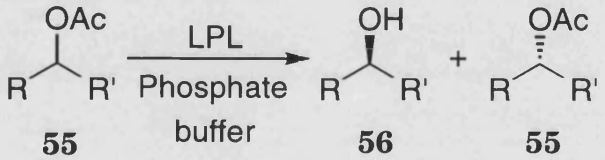
In the previous Chapter the resolution of allylic acetates was discussed along with the racemisation techniques that were to be employed. Due to the complications of the racemisation protocol it was decided to look at a different set of substrates and apply an appropriate racemisation technique to them.

In this Chapter the processes and stages towards the *dynamic resolution* of some non-allylic acetates of the general structure shown below , **Scheme 39** will be outlined.



Scheme 39.

Kim and Cho [29] studied the lipoprotein lipase, from *Pseudomonas sp.* (LPL) catalysed hydrolysis of some secondary acetates, **Scheme 40**, with a view of defining a model of the active site.



R	R'	(OH) ee %	(OH) Config.	Rate[a]
CH_3	Ph	> 98	R	100
CH_3	CH_2Ph	> 98	R	8
CH_3	CH_2CH_2Ph	55	R	8
CH_3CH_2	Ph	> 98	R	7
$ClCH_2$	CH_2Ph	88	S	9
$ClCH_2$	CH_2OPh	90	S	34
$BrCH_2$	CH_2Ph	75	S	9

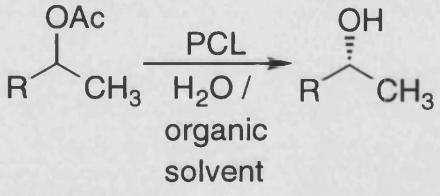
a) Values are relative to each other.

Scheme 40.

In this study it was found that as the size of R increased the reactivity of the substrate decreased rapidly, the substrate will become completely inactive if R is bulkier than n-Pr.

It was also found that the enantioselectivity of the enzyme was improved if R' was a large hydrophobic substituent, e.g. Ph. If the chain length of R' is increased then the observed enantioselectivity displayed by the enzyme decreased slightly.

Naoshima *et al* [30] studied the enzymatic hydrolysis of a series of alkan-2 and alkan-3-ols using PCL in the presence of organic solvents, **Scheme 41.**



57 R= $CH_3(CH_2)_{10}$ **59** R= $CH_3(CH_2)_{10}$
58 R= $CH_3(CH_2)_5$ **60** R= $CH_3(CH_2)_5$

R	Organic Solvent	Time hrs	Conv. %	(OH) ee %
$CH_3[CH_2]_{10}$	none	24	37	76
	acetone	19	32	96
	methanol	47	39	83
	hexane	84	30	69
$CH_3[CH_2)]_5$	none	14	46	40
	acetone	20	33	81
	methanol	22	43	69
	hexane	47	33	63

Scheme 41.

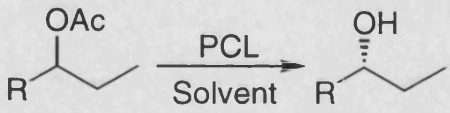
Here it was shown that the resolution of 2-acetoxytridecane **57** in a water system was hydrolysed by PCL in low enantiomeric purity, 76%. With the addition of an organic solvent the enantioselectivity

increased dramatically, an optimum system for the resolution involves an acetone / phosphate buffer solvent with the alcohol **59** being resolved at 96% enantiomeric excess.

The acetate 2-acetoxyoctane **58**, was hydrolysed in a water system with PCL to yield the corresponding alcohol **60** with a very low enantiomeric excess, 40%. When hydrolysed in an organic / aqueous system the enantioselectivity of the resolution increased to a maximum of 81% ee in a water / acetone system.

These results also suggest that the long chain acetate can be resolved with greater enantioselectivity than their short chained analogues.

The authors also studied the effects of a two phase solvent system on the hydrolysis of 3-acetoxyalkanes, **Scheme 42**.



61 R= $CH_3(CH_2)_9$ **63** R= $CH_3(CH_2)_9$
62 R= $CH_3(CH_2)_4$ **64** R= $CH_3(CH_2)_4$

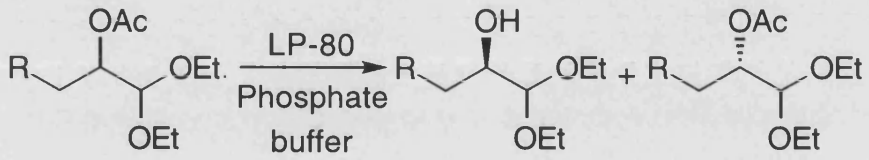
R	Organic Solvent	Time hrs	Conv. %	(OH) ee %
$CH_3[CH_2]_9$	none	71	45	56
	acetone	49	46	80
	methanol	72	41	54
	hexane	95	33	55
$CH_3[CH_2]_4$	none	58	37	63
	acetone	54	32	81
	methanol	32	43	72
	hexane	144	40	69

Scheme 42.

As shown in **Scheme 42** the analogous lipase catalysed resolution of the two 3-acetoxyalkanes **61**, **62** was performed in organic / aqueous solvent systems. Each hydrolysis of the acetates **61** and **62**

proceeded with higher enantioselectivity in an acetone / phosphate buffer system than in other solvent systems, resulting in (R)-alcohol **63** of 80% ee and the (R)-alcohol **64** in 81% ee.

Wong *et al* [31] investigated the LP-80 lipase catalysed hydrolysis of 2-acetoxy-3-fluoro and 2-acetoxy-3-chloropropanal diethyl acetals **64**, **65**, **Scheme 43**.



64 R= F
65 R= Cl

(R)-66 R= F
(R)-67 R= Cl

(S)-64 R= F
(S)-65 R= Cl

	R	(R)-OH ee %	Yield %[a]
64	CH_2F	> 98	92
65	CH_2Cl	> 98	92

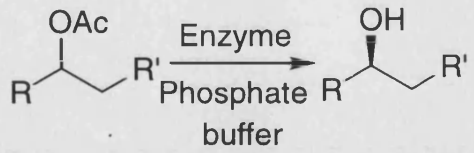
a) Calculated yield of the theoretical maximum.

Scheme 43.

In this example it was shown that these two acetals are readily resolved with very high enantioselectivity using LP-80 lipase in an aqueous buffered system. The authors also extended this application of LP-80 to the *kinetic resolution* of 2-acetoxy-1-(benzyloxy)-3-chloropropane **68**, and two other derivatives **69** and **70**, **Scheme 44**.

These three substrates were screened in an aqueous phosphate buffer with LP-80 and *Porcine pancreatic* lipase (PPL) and all three substrates were successfully resolved in good enantiomeric excesses and yields.

The author demonstrated the use of hydrolytic enzymes in the preparation of highly enantiomerically pure epoxy aldehydes and epoxy alcohols.

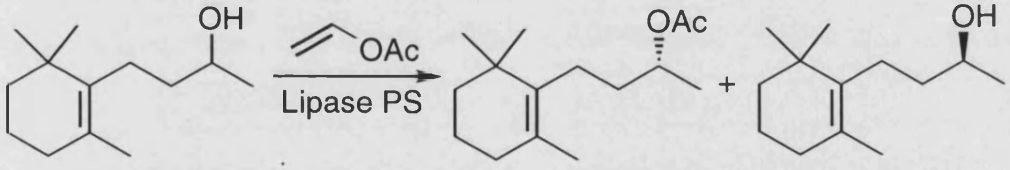


68 R= CH_2Cl R'= OCH_2Ph
69 R= CH_3 R'= OTs
70 R= CH_2CHCH_2O R'= OTs
71 R= CH_2Cl R'= OCH_2Ph
72 R= CH_3 R'= OTs
73 R= CH_2CHCH_2O R'= OTs

Substrate	Enzyme	Conv. %	(OH) ee %
68	LP-80	60	63
68	PPL	25	92
68	PPL	60	75
69	LP-80	-	90
70	LP-80	-	94

Scheme 44.

Ohta *et al* [32] used an interesting two stage enzymatic acetylation followed by an enzymatic hydrolysis to afford a precursor in the total asymmetric synthesis of Dyhydroedulan II, a natural product found in butterflies, **Scheme 45**.



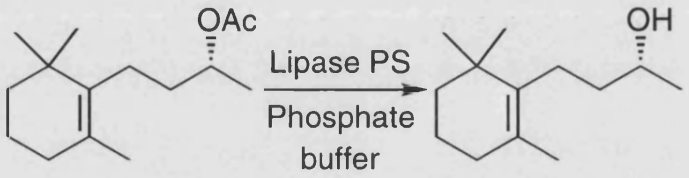
74 **(R)-75** **(S)-74**

Scheme 45.

When racemic **74** was treated with Lipase PS, from *Pseudomonas sp.* and vinyl acetate at 22 °C for 24 hours (R)-acetate **75** was isolated in a ~50% yield at 96% enantiomeric excess. The unreacted (S)-alcohol **74** was also recovered in a ~50% yield and its enantiomeric excess was found to be 94%. For their strategy to succeed the opposite enantiomer of alcohol was required, i.e. they obtained the (S)-enantiomer but (R)-enantiomer was required.

In order to invert the stereocentre of the product alcohol Mitsunobu [33] conditions were applied to yield the alcohol with the opposite (R)-configuration in 93% yield and 94% enantiomeric excess.

In order not to 'waste' his chiral acetate **75** he subjected this acetate to an enzymatic hydrolysis using lipase PS in an aqueous solution in the hope of increasing his already high enantiomeric excess by leaving any unwanted (S)-enantiomer behind, **Scheme 46**.



(R)-75 **(R)-74**

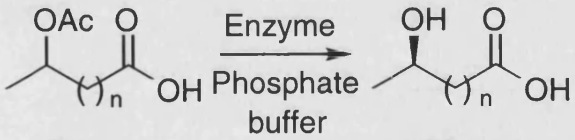
Scheme 46.

In practice the hydrolysis proceeded smoothly to afford the (R)-alcohol **74** in a 96% yield, of which the enantiomeric excess was almost the same as that of the starting material.

The alcohol **74** possessing the desired configuration (R) was obtained in a 68% overall yield with very high enantiomeric excess, 96%. The authors were able to perform these transformations using over 15 g of the racemic starting material **74**.

Scilimati, Ngooi, and Sih [34] investigated the enzymatic hydrolysis of a series of acetoxyalkanoic acids **76**, and **78**, **Scheme 47**.

A number of enzymes from *Pseudomonas sp.* were investigated for their capabilities in catalysing the hydrolysis of the racemic acetoxyalkanoic acids of varying chain lengths and it was found that all of the lipases displayed rather low enantioselectivity towards these substrates.



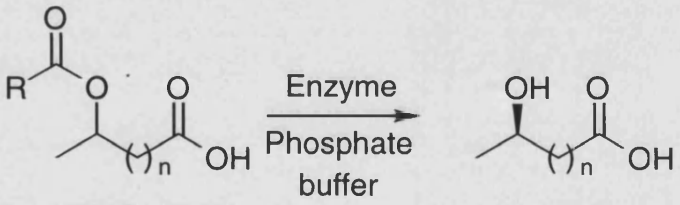
76 n= 1
78 n= 5

77 n= 1
79 n= 5

n	Enzyme	Time hrs	Conv. %	(R)-(OH) ee %
1	AK	14	41	10
1	K-10	24	17	50
5	AK	100	42	57
5	K-10	16	26	45

Scheme 47.

In an attempt to increase the selectivity of the enzymes longer chain esters were synthesised, n-butyl **80** and n-hexyloxyalkenoic **81** acids, **Scheme 48**.



80 R= C_3H_7
81 R= C_5H_{11}

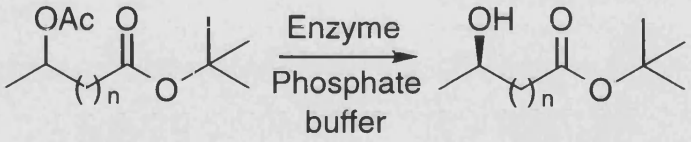
79

n	R	Enzyme	Time hrs	Conv. %	(R)-(OH) ee %
5	n-C_3H_7	AK	12	32	65
5		K-10	13	17	86
5	n-C_5H_{11}	AK	3	50	53
5		K-10	4	50	64

Scheme 48.

Although no improvement in enantioselectivity was noted with the AK lipase a moderate improvement in enantioselectivity was observed with the K-10 lipase.

In further investigations the authors converted the acids into t-butyl ester functions **82, 83** and noted that again there was a marked increase in the enantioselectivity displayed by both the enzymes, **Scheme 49**.



82 n= 1
83 n= 5

84 n= 1
85 n= 5

n	Enzyme	Time hrs	Conv. %	(R)-(OH) ee %
1	AK	15	41	99
1	K-10	16	38	99
5	AK	3	31	99
5	K-10	5	28	99

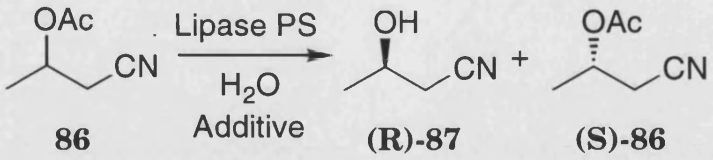
Scheme 49.

Itoh *et al* [35] investigated the effects of crown ether type additives on the enantioselectivity displayed by lipase PS during the enzymatic hydrolysis of 2-acetoxybutanenitrile **86, Scheme 50**.

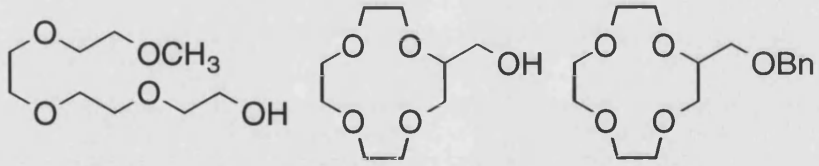
Here it can be seen that only HM-12C4 has an incremental effect on the stereoselectivity displayed by Lipase PS towards the hydrolysis of the substrate **86**; TEM and BnM-12C4 did not affect the overall enantiomeric excess of the final (R)-alcohol **87**.

In a later paper from his group [36] Itoh investigated the effects of over twenty seven crown ethers upon the stereoselectivity of lipase SP on 2-acetoxybutanenitrile **86**. A selection of the results are shown **Scheme 51**.

Employing a crown ether additive is an attractive and easy to employ alternative to modification of substrate in order to increase the stereoselectivity of an enzyme.



86 **(R)-87** **(S)-86**



TEM HM-12C4 BnM-12C4

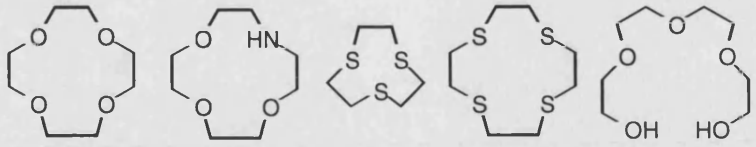
Additive	mol %	Time hrs.	Conv. %	(R)-OH ee %
none	-	31	31	76
TEM	33	24	42	73
BnM-12C4	33	24	42	74
HM-12C4	10	19	35	76
HM-12C4	33	20	40	80
HM-12C4	50	19	45	77
HM-12C4	100	18	44	81

Scheme 50.

Adje, Breuilles, and Uguen [37] treated an alkynic diacetate **93** with PFL in a phosphate buffer to yield the mono-acetate **94**, **Scheme 52**.

The diacetate, **93** was treated with PFL in a phosphate buffer and maintained at pH 7 over 2 weeks at 18 °C. The only product isolated from this reaction was the (2R, 5S) monoacetate **94** in 83% yield, 97 % enantiomeric excess.

Though the reaction time is very long this is a rare application of enzyme mediated hydrolysis applied to alkynic substrates.

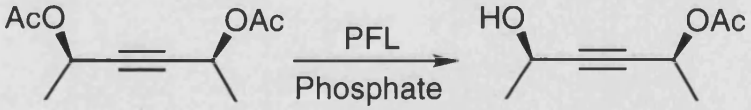


88 **89** **90** **91** **92**

Additive	mol %	Time hrs	Conv. %	(R)-OH ee %
88	38	14	18	80
89	38	14	45	79
90	38	14	50	74
91	38	14	59	71
92	38	14	22	85

Scheme 51.

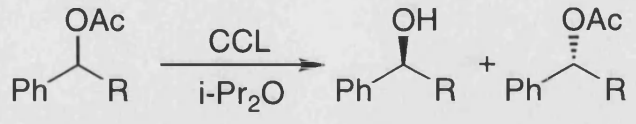
Bevinakatti *et al* [38] described the resolution of secondary alcohols **95** using a lipase in diisopropyl ether, **Scheme 53**.



93 **(R,S)-94**

Scheme 52.

Here it was demonstrated that the methyl ester **95** (R= CO_2CH_3) was not resolved efficiently by the enzyme in diisopropyl ether. After 23 days the reaction had only progressed to 25% conversion but the final enantiomeric excess was very good (98% ee). The butyl ester **95** (R= CO_2Bu) was resolved with a greater efficiency, 45% conversion with 92% enantiomeric excess. The nitrile analogue **95** (R= CN) was resolved with a good stereoselectivity ~ 77% ee, but the resolution of the chlorinated analogue **95** (R= CH_2Cl) was very poor.



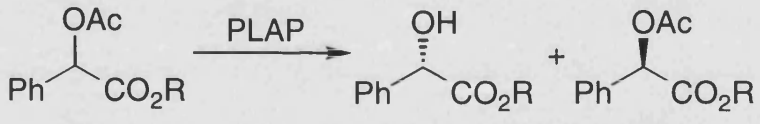
95 **96** **95**

R	Time days	Conv. %	(OH) ee %	(OAc) ee %
CO_2CH_3	23	25	98	29
CO_2Bu	7	45	92	82
CN	2	38	77	-
CN	4	40	67	-
CH_2Cl	19	42	22	19

Scheme 53.

In a more recent paper Basavaiah and Krishna [39] also conducted studies into the *kinetic resolution* of methyl mandelate and some of its derivatives using Pig Liver acetone powder (PLAP), **Scheme 54**.

The hydrolysis of acetoxy methyl mandelate **97** (R= CH_3) was carried out in various conditions and it was found that a two phase medium produced the desired hydroxy ester **98** (R= CH_3) in a 75% enantiomeric excess. The authors also noted that the methyl ester functionality remained completely intact.



97 **98** **97**

R	Time hrs	Conv. %	(OH) ee %	(OAc) % ee
CH_3	5	48	75	70
CH_3CH_2	8	41	47	35
i-Pr	17	47	55	50
t-Bu	17	34	80	49
Cyclohexyl	60	31	23	71

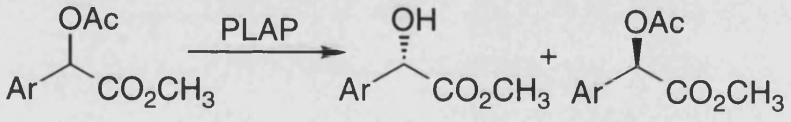
Scheme 54.

From these results it is apparent that methyl and t-butyl esters of mandelic acid provide better selectivities compared to the other three ester groups in enzymatic hydrolysis using PLAP. Also acetoxy methyl mandelate is hydrolysed faster by PLAP compared to the other substrates studied.

The authors also studied the effects of substitution on the aromatic ring on selectivity, **Scheme 55**.

A series of substituted acetoxy methyl mandelate derivatives was prepared, **97** (Ar= 2-OCH_3Ph, 4-CH_3Ph, 4-OCH_3Ph) and treated with PLAP in the two-phase soulution. The hydrolysis of **97** (Ar= 2-OCH_3Ph) with PLAP produced the alcohol **98** (Ar= OCH_3Ph) in 55% enantiomeric excess and all other substrates showed similar selectivities.

The authors concluded that substitution on the aromatic ring increased the rates of hydrolysis but resulted in poorer enantioselectivity.



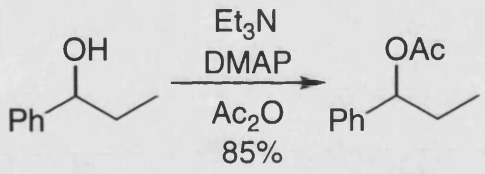
97 **98** **97**

Ar	Time hrs	Conv. %	(OH) ee %
2-OCH_3Ph	2	43	55
4-CH_3Ph	4	45	26
4-OCH_3Ph	3	44	57

Scheme 55.

3.2 Preparation of acetates.

1-Phenyl-1-acetoxy propane **100** was easily synthesised from the commercially available 1-phenylpropan-1-ol **99**, **Scheme 56**.

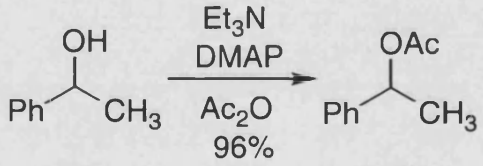


99 **100**

Scheme 56.

Acetylation carried out using acetic anhydride proceeded smoothly and following flash chromatography yielded acetate **100** in 85% yield. Confirmation of the formation of the acetoxy moiety came from the appearance of a methyl singlet at 2.21 ppm in the ^{1}H NMR spectrum.

1-phenethyl acetate **102** was similarly synthesised from the commercially available 1-phenethyl alcohol **101**, **Scheme 57**.



101 **102**

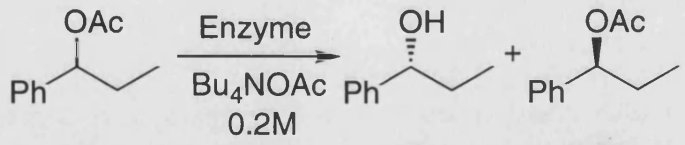
Scheme 57.

Treatment of 1-phenethyl alcohol with triethylamine, acetic anhydride and a catalytic amount of DMAP resulted in 1-phenethyl acetate **102** being isolated in 96% yield, following flash chromatography.

3.3 Enzymatic hydrolysis of acetates.

Before a suitable racemisation process can be developed the enzymatic *kinetic resolution* of the substrates must first be investigated.

1-Phenyl-1-acetoxy propane **100** was screened with our collection of enzymes in tetrabutyl ammonium acetate buffer, **Scheme 58**.



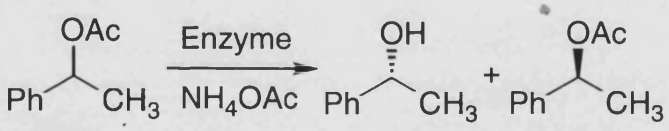
100 **99** **100**

Enzyme	Time	Conv.	(OH)[a]	(OAc)[a]
	days	%	ee %	ee %
PFL	2	48	99	75
PCL	2	51	99	79
CAL	3	12	1	14

Reactions carried out at 55 °C. a) Enantiomeric excess determined by HPLC analysis (Diacel Chiracel OD column, 99:1 n-hexane:IPA).

Scheme 58.

We were very pleased by these initial results and applied these enzymes in the hydrolysis of 1-phenethyl acetate **102**, **Scheme 59**.



102 **101** **102**

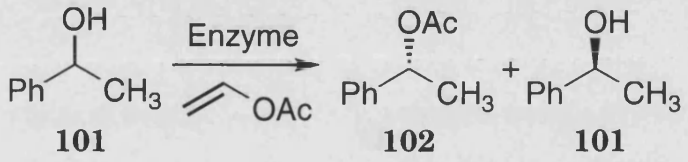
Enzyme	Time	Conv.	(OH)[ab]	(OAc)[a]
	days	%	ee % / Config.	ee %
PCL	5	46	95 / (R)	87
PFL	6	38	87 / (R)	72

Reactions carried out at 50 °C. a) Enantiomeric excess determined by HPLC analysis (Diacel Chiracel OD column, 99:1 n-hexane:IPA). b) Determined by comparison of HPLC spectra to a sample of known stereochemistry.

Scheme 59.

Once again these results were very pleasing and were comparable to the results of Kim and Cho using Lipoprotein lipase.

Next a racemisation protocol was required. In order to obtain the chiral acetate that was required for studies into the racemisation, the enzymatic acetylation of the corresponding alcohol was investigated, **Scheme 60**.



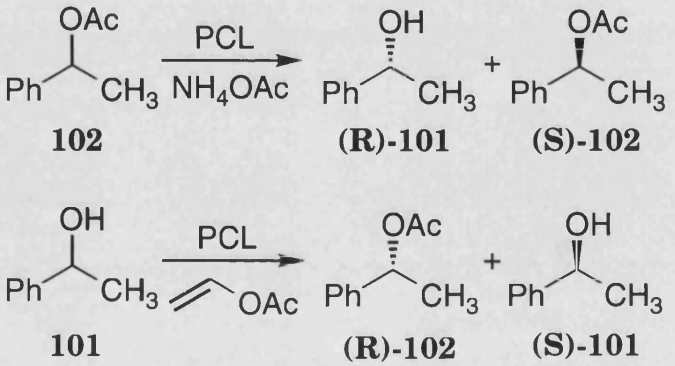
101 **102** **101**

Enzyme	Time	Temp	Conv.	(OAc)[ab]	(OH)[a]
	days	°C	%	ee % / Config.	ee %
PCL	4	38	48	96 / (R)	88
PFL	5	25	41	97 / (R)	84

a) Enantiomeric excess determined by HPLC analysis. b) Determined by HPLC analysis.

Scheme 60.

Once again the results were very pleasing and following separation of the unreacted alcohol (S)-**101** from the acetate, enantiomerically pure acetate (R)-**102** was available to us in very good yield, 47%.



102 **(R)-101** **(S)-102**

101 **(R)-102** **(S)-101**

Scheme 61.

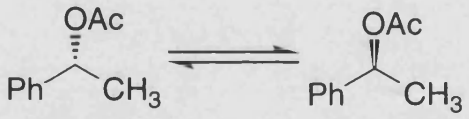
We were now able to prepare either enantiomer of alcohol **101** or acetate **102** in good yields and enantiomeric excess, **Scheme 61**.

3.4 Racemisation of 1-phenethyl acetate.

Our initial strategy for the racemisation of **102** was the deprotonation of the α-proton using mildly basic conditions [40]. The racemisation should be carried out in a solution that was basic enough to deprotonate this proton without affecting the activity or stereoselectivity of the enzyme.

All racemisation studies were carried out using enantiomerically pure commercially available (R)-phenethyl acetate **102**.

Preliminary experiments involved the treatment of (R)-acetate **102** in a basic solution, **Scheme 62**.



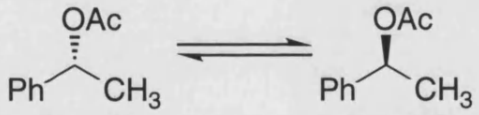
(R)-102 **(S)-102**

Conditions	Temp °C	Time days	(OAc) ee %[a]
Et_3N (15 µl), Petrol (2 ml)	38	3	99
KOH (0.2M, 2 ml)	40	6	99

a) Final enaniomeric excess determined by HPLC analysis.

Scheme 62.

Unfortuntely no racemisation was seen using these conditions. Next we attempted racemisation using oxaphillic metals as catalysts. Here enantiomerically enriched acetate **102** was treated with an oxophilic metal in an aqueous acetate rich environment. It was envisaged that the metal catalyst would co-ordinate to the acetate group and weaken the C-O bond and allow an incoming acetate to displace the original acetate via an S_N2 mechanism, hence racemisation would occur, **Scheme 63**.



(R)-102 **(S)-102**

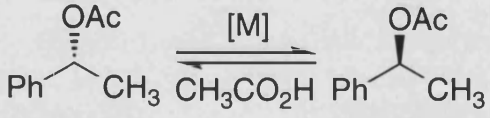
Conditions	Temp. °C	Time days	(OAc)[a] ee %
$Yb(OTf)_3$ / Bu_4NOAc (0.1M, 1 ml)	40	2	99
$Sc(OTf)_3$ / Bu_4NOAc (0.1M, 1.5 ml)	40	6	97
$Yb(OTf)_3$ / Bu_4NOAc (0.1M, 1.5 ml)	40	6	99

a) Final enantiomeric excess determined by HPLC analysis.

Scheme 63.

Unfortunately no racemisation took place but we carried out further continuous S_N2 displacement reactions of the acetate catalysed by Lewis acids.

Here enantiomerically enriched acetate **(R)-102** was treated with acetic acid in the presence of a lewis acid, **Scheme 64**.



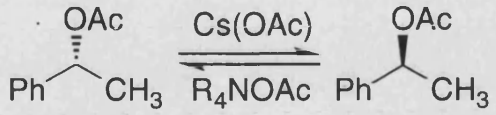
(R)-102 **(S)-102**

Conditions	Temp. °C	Time days	(OAc)[a] ee %
$NiCl_2$ / CH_3CO_2H (0.1M, 1 ml)	25	4	99
$ZnCl_2$ / CH_3CO_2H (0.1M, 1 ml)	25	4	99
$PdCl_2$ / CH_3CO_2H (0.1M, 1 ml)	30	5	98
PdO_2 / CH_3CO_2H (0.1M, 1 ml)	30	5	95

a) Final enantiomeric excess determined by HPLC analysis.

Scheme 64.

Once again we were unable to racemise the starting material using acetic acid. We next attempted to racemise by using a very acetate rich environment. In these experiments enantiomerically enriched acetate **(R)-102** was treated with caesium acetate in a quaternary ammonium acetate buffer solution, **Scheme 65**.



(R)-102 **(S)-102**

R	Conditions[a]	Temp °C	Time days	(OAc)[b] ee %
Butyl	0.2M, 0.8 ml	55	3	99
Butyl	0.1M, 1.5 ml	40	7	97
Butyl	0.1M, 1.5 ml	80	4	95
H	0.1M, 0.8 ml	56	3	99
Butyl	sat. DCM 2 ml	40	3	99

a) 10 mol % caesium acetate used throughout. b) Final enantiomeric excess determined by HPLC analysis.

Scheme 65.

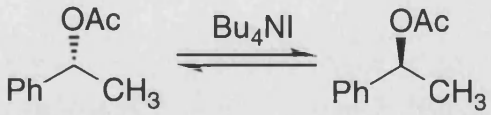
We were still unable to acheive racemisation of the substrate. Next we attempted the S_N2 displacement of the acetate group with an iodide [41] which could in turn be displaced by another acetate which would result in racemisation of the substrate, **Scheme 66.**

It was disappointing to find that these conditions were unable to racemise the substrate.

From this data it is apparent that the racemisation of this type of substrate was going to prove difficult so we decided to alter the substrate in order to facilitate a useful racemisation protocol.

We envisaged that if the carbon was more electrophilic then S_N2 displacement would become more viable. If the methyl group is substituted for an ester function then the S_N2 transition state could

be stabilised by electronic donation to the carbonyl π^* orbital, **Scheme 67**.



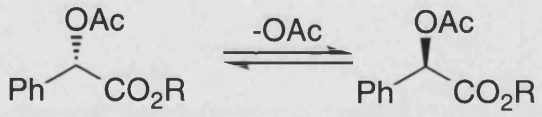
(R)-102 **(S)-102**

Conditions	Temp. °C	Time days	(OAc)[a] ee %
Bu_4NI sat. DCM (0.5 ml)	35	2	99
Bu_4NI sat. DCM (0.5 ml)	50	1	99
Bu_4NI sat.DCM (0.5 ml) / Bu_4NOAc sat DCM (0.5 ml)	35	2	99
Bu_4NI sat.DCM (0.5 ml) / Bu_4NOAc sat DCM (0.5 ml)	50	1	99

a) Final enantiomeric excess determined by HPLC analysis.

Scheme 66.

Here we see the ester function fulfilling two roles. Firstly withdrawing electron density from the carbon making nucleophilic attack more viable, and secondly allowing the stabilisation of the transition state.



Scheme 67.

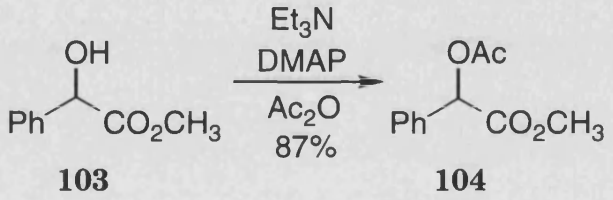
Previous work by the groups of Basavaiah and Bevinakatti reported the enzymatic hydrolysis of acetoxy methyl mandelate with good enantiomeric excess and we aimed to emulate their results and apply the above S_N2 racemisation to the enzymatic *kinetic resolution* to acheive a *dynamic resolution*.

3.5 Synthesis of acetoxy methyl mandelate 104.

Synthesis of the starting material involved simple acetylation of commercially available methyl mandelate **103** in triethylamine, acetic anhydride, and a catalytic amount of DMAP, **Scheme 68.**

The reaction proceeded very smoothly and went to completion in around 1.5 hours. Following an aqueous work up and flash chromatography a pure sample of acetate **104** was obtained in 87% yield.

Analysis of the ^1H NMR spectra gave comparable results to literature values.



103 **104**

Scheme 68.

3.6 Enzymatic *kinetic resolution* of acetoxy methyl mandelate.

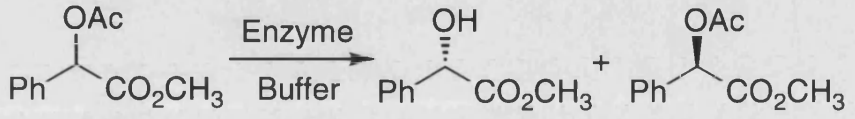
Bevinakatti *et al* resolved the acetate of methyl mandelate in a diisopropyl ether system using CCL in 25% conversion over 23 days with 98% ee of the (R)-alcohol. Basavaiah and co workers treated the acetate of methyl mandelate with PLAP in an unspecified two phase system to yield (S)-alcohol in 75% ee over 5 hours at 48% conversion.

Though the first example produced the desired alcohol in very good enantiomeric excess, reaction time is too long and the conversion very low. The second example details the very quick hydrolysis but with a slightly low enantiomeric excess of the desired alcohol.

We felt that the resolution of acetoxy methyl mandelate could be improved to provide a *kinetic resolution* with very high enantioselectivity combined with short reaction time. Initial

screenings were carried out using PFL and PCL in two different buffer systems, **Scheme 69**.

As detailed below the hydrolysis using PFL in either NH_4OAc or Bu_4NOAc buffers gave a highly selective hydrolysis in moderate reaction time to yield the (S)-alcohol **103**.



104 **(S)-103** **(R)-104**

Enzyme	Buffer	Conv. %	(S)-(OH)[a] ee %	(OAc)[a] ee %
PFL	NH_4OAc	47	99	78
PCL	NH_4OAc	35	99	80
PFL	Bu_4NOAc	46	99	98
PCL	Bu_4NOAc	38	99	69

Reactions carried out at 40 °C over 3 days. a) Enantiomeric excess determined by HPLC analysis (Diacel Chiracel OD or OJ column, 99:1 n-hexane:IPA).

Scheme 69.

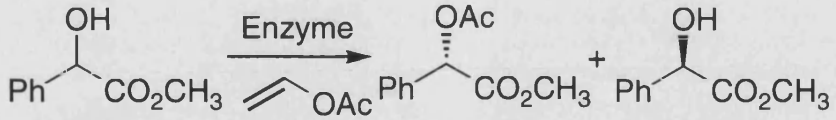
With the enzymatic hydrolysis in hand we required enantiomerically enriched acetate for racemisation studies.

As before we screened the substrate against a selection of enzymes, including PFL and PCL, to catalyse the reverse reaction, i.e. the acetylation of **103**, **Scheme 70.**

Once again PFL and PCL gave very pleasing results. PFL catalysed hydrolysis gave (S)-acetate **104** with 82% ee at 37% conversion, and PCL 72% ee at 29% conversion.

3.7 Racemisation of acetoxy methyl mandelate.

Initial experiments carried out towards the racemisation of **(S)-104** centralised around the basis of a continual S_N2 displacement of the acetate by another incoming acetate.



103 **(S)-104** **(R)-103**

Enzyme	Time days	Conv. %	(S)-(OAc)[a] ee %	(OH)[a] ee %
PFL	5	37	82	77
PCL	6	29	72	79
CAL	7	34	47	81
CCL	6	34	29	64

Reactions carried out at 38 °C. a) Enantiomeric excess determined by HPLC analysis.

Scheme 70.

First experiments were carried out on acetylated (Et_3N, Ac_2O, and DMAP) commercially available (S)-methyl mandelate (99% ee) in a sealed tube, **Scheme 71**.

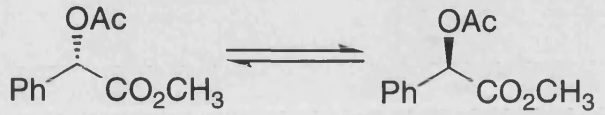
These preliminary results were very encouraging. Here we see that the acetate **(S)-104** can be racemised using Bu_4NOAc in DCM (entry 1, **Scheme 71**) at 80 °C over 3 days. The enantiomeric excess of the acetate does not fall at an appreciable rate after this amount of time (entry 2, **Scheme 71**).

With the addition of caesium acetate (entry 5, **Scheme 71**) it appears that the racemisation will take place at a lower temperature but this may be due to the presence of tetrabutyl ammonium acetate in such high concentration.

Though we are able to racemise the substrate these conditions are too extreme to be tolerated by the enzyme. For an ideal system the

racemisation should take place in an aqueous system at around 50 °C, the optimum temperature for PFL.

We know that tetrabutyl ammonium acetate is able to carry out the racemisation as a saturated solution in organic solvent but will this racemisation take place in aqueous solutions ?



(S)-104 **(R)-104**

Entry	Conditions	Time days	(OAc)[a] ee %
1	Bu_4NOAc sat DCM (0.8 ml) 80 °C	3	6
2	Bu_4NOAc sat DCM (0.8 ml) 80 °C	4	3
3	Bu_4NOAc sat DCM (0.5 ml) / Et_3N (10 µl) 80 °C	2	18
4	Bu_4NOAc sat DCM (0.5 ml) / Bu_4NI sat DCM (0.5 ml) 80 °C	3	99
5	Bu_4NOAc sat DCM (0.8 ml) / Cs(OAc) (20 mol%) 55 °C	3	7

Racemisation experiements were carried out using the acetate of commercially available (S)-methyl mandelate (99% ee). a) Final enantiomeric excess determined by HPLC analysis.

Scheme 71.

We also wanted to explore the influence of a secondary acetate salt in the reaction. As stated above the presence of 20 mol% of Cs(OAc) in the reaction allows the reaction to be carried out at 55 °C, but is this affecting the racemisation as we know that tetrabutyl ammonium acetate can racemise on its own (entry 1, **Scheme 71**).

A series of reactions were carried out using solutions of quaternary ammonium acetate salts in the presence of an additional acetate salt (20 mol%) in sealed tubes at 55 °C unless otherwise stated, **Scheme 72**.

Here it is demonstrated that the substrate is successfully racemised in an aqueous solution at 55 °C.

(S)-104 **(R)-104**

Entry	Conditions	Time days	(OAc)[a] ee %
1	NH_4OAc (0.1M, 1 ml) Cs(OAc)	1	99
2	NH_4OAc (0.1M, 0.5 ml) / Cs(OAc) Bu_4NOAc (0.1M, 0.5 ml)	1	99
3	Bu_4NOAc (0.2M, 0.8 ml) Cs(OAc)	2	25
4	Bu_4NOAc (0.3M, 0.8 ml) Cs(OAc)	2	27
5	Bu_4NOAc (0.4M, 0.8 ml) Cs(OAc)	2	26
6	Bu_4NOAc (0.5M, 0.8 ml) Cs(OAc)	2	29
7	Bu_4NOAc (0.2M, 0.8 ml) Cs(OAc)	2	29
8	Bu_4NOAc (1.0M, 0.8 ml) Cs(OAc)	2	30
9	Bu_4NOAc (0.1M, 0.8 ml) $Pd(OAc)_2$ 80 °C	2	13

Racemisation experiements were carried out using the acetate of commercially available (S)-methyl mandelate (99% ee). a) Final enantiomeric excess determined by HPLC analysis.

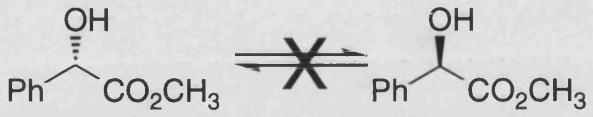
Scheme 72.

Ammonium acetate is unable to racemise the substrate and this may be due to the size of the cation. If the cation is large then once in solution the positive cation can be solvated by water molecules and thus be more dissociative allowing more 'free' acetate to take part in the racemisation. If the cation is smaller then it will not be as solvated and so less 'free' acetate is available in the solution.

Treatment with tetrabutyl ammonium acetate and 20 mol% Cs(OAc), (entries 3-9, **Scheme 72**) will racemise the substrate at 55 °C. The enantiomeric excess fell from 99% to ~ 28% ee over a period of 2 days.

In order to obtain *dynamic resolution* it is necessary for the hydrolysed product, methyl mandelate **103** to be unaltered by the racemisation. If the alcohol is racemised then all enantioselectivity demonstrated by the enzyme will be wasted.

(S)-methyl mandelate **103** was treated with the racemisation conditions, **Scheme 73**.



(S)-103 **(R)-103**

Entry	Conditions	Time days	(OH)[a] ee %
1	Bu_4NOAc (sat DCM, 1 ml) / Cs(OAc) 80 °C	2	99
2	Bu_4NOAc (sat DCM, 0.8 ml) / Cs(OAc) 50 °C	3	99
3	NH_4OAc (0.1M, 1 ml) / Cs(OAc) 55 °C	1	99
4	Bu_4NOAc (0.1M, 1 ml) / Cs(OAc) 55 °C	1	99

Racemisation experiements were carried out using commercially available (S)-methyl mandelate (99% ee). a) Final enantiomeric excess determined by HPLC analysis.

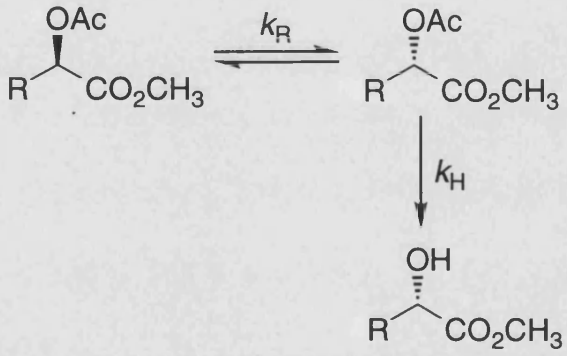
Scheme 73.

No racemisation of the alcohol **(S)-103** was observed using any of these systems. If very acetate rich environments were used (entries 1 and 2, **Scheme 73**) then racemic acetate was produced in the reaction.

3.8 *Dynamic resolution* of acetoxy methyl mandelate.

With our *kinetic resolution* and selective racemisation in hand the two processes have to be combined in order to acheive a *dynamic resolution*. Initially this would appear to be a simple task but when considered carefully certain facts come to light.

During a *dynamic resolution* we have two reactions taking place at the same time in the same place. We have to consider the rates of each process taking place, **Scheme 74**.



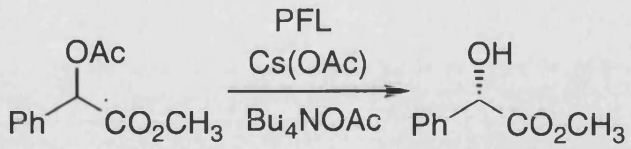
Scheme 74.

Here we have the rate of racemisation, k_R and rate of hydrolysis, k_H. If the rate of racemisation is too slow, i.e. $k_R < k_H$ then there will be a build up of wrong enantiomer of acetate and conversion will not be complete without the loss of selectivity and enantiomeric excess. For an ideal *dynamic resolution* the rate of racemisation should be equal or slightly quicker than the rate of hydrolysis, i.e. $k_R = k_H$. This will ensure that there is a constant supply of the correct enantiomer of acetate and the reaction can go to completion.

The results for the *dynamic resolution* of acetoxy methyl mandelate **104** are shown, **Scheme 75**.

Reactions were carried out in acetate buffer and immobilised PFL was utilised. 20 mol% of Cs(OAc) was used in all the reactions.

The reaction carried out at 80 °C failed due to the higher temperature. At this temperature the support on which the enzyme was immobilised (Sol Gel AK) decomposed and reaction failed.



104 **(S)-103**

Conditions	Conv. %	Time days	Temp. °C	(S)-(OH)[a] ee %	(OAc)[a] ee %
0.1M Bu_4NOAc	55	6	55	99	99
0.2M Bu_4NOAc	23	2	55	99	79
0.2M Bu_4NOAc	38	6	55	99	83
0.3M Bu_4NOAc	32	2	55	99	79
0.2M Bu_4NOAc	-	2	80	-	-
0.2M Bu_4NOAc / Bu_4NOAc sat. DCM	22	2	50	99	53

a) Enantiomeric excess determined by HPLC analysis.

Scheme 75.

It is clear from the results specified that the two processes (racemisation and hydrolysis) were occurring out of 'sync'. Since the starting material, acetate **104** is enantiomerically enriched after the reaction is stopped implies that the racemisation is taking place at a rate significantly slower than the rate of hydrolysis, i.e. $k_R < k_H$ and so there is a build up of unprocessable acetate and reaction stops.

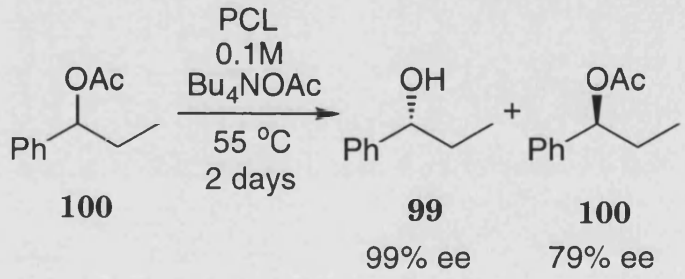
If 0.2M Bu_4NOAc is used then it is apparent that hydrolysis is very hampered and after 6 days conversion only reaches 38%. Conducting the experiment in 0.1M Bu_4NOAc provides a conversion of 55% after 2 days. This may imply that the enzyme is being denatured by the amount of acetate in the system and is unable to process the unhydrolysed substrate.

Also to be considered is that when the alcohol is subjected to the racemisation conditions a small amount of acetate is produced via a S_N2 mechanism. If the product alcohol is of (R)-configuration then the enzyme is processing the (R)-acetate. The product (R)-alcohol is converted to (S)-acetate via the S_N2 mechanism and if the racemisation is occurring at a slower rate than the enzymatic hydrolyisis then the (S)-acetate will build up in the reaction mixture and will not be processed by the enzyme. This would result in the observed results and account for the breakdown of the *dynamic resolution*.

Unfortunately due to time constraints no further work was able to be carried out on this system.

3.9 Conclusions.

In this chapter the enzymatic *kinetic resolution* of 1-phenyl-1-acetoxy propane **100** has been described, **Scheme 76**.

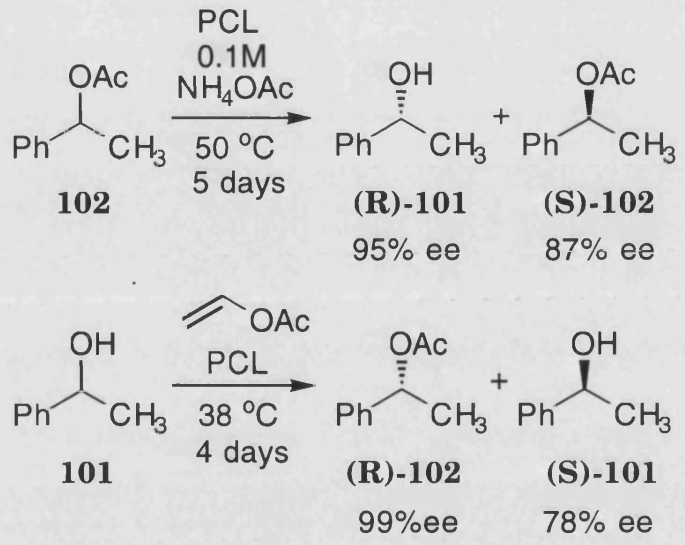


Scheme 76.

1-Phenyl-acetoxy propane **100** was stirred with PCL in tetrabutyl ammonium acetate buffer over 2 days at 55 °C. 1-Phenyl propan-1-ol **99** in 99% ee, and 1-phenyl-acetoxy propane **100** in 79% ee at 55% conversion was isolated. A highly selective hydrolysis yielding both acetate **100** and alcohol **99** in very good enantiomeric excess.

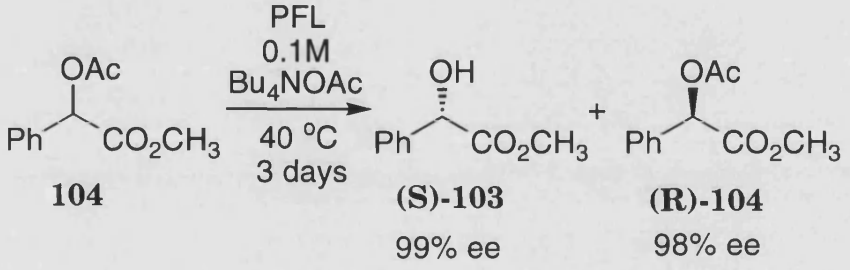
Also described was the PCL catalysed hydrolysis and acetylation of phenethyl alcohol **101** and its acetate **102**, **Scheme 77**, enabling the

formation of either enantiomer of either product in good enantiomeric excess.



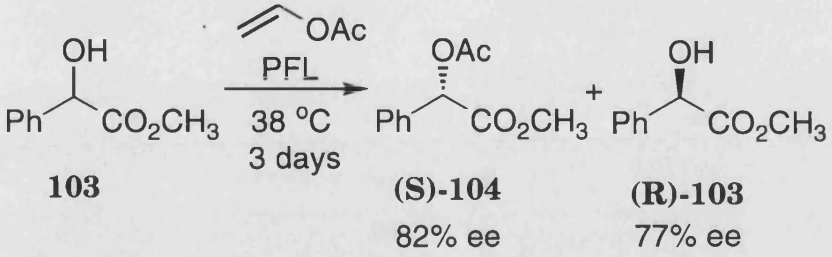
Scheme 77.

Also the enzymatic hydrolysis of acetoxy methyl mandelate **104** with PFL in tetrabutyl ammonium acetate at 38 °C over 3 days to yield (S)-methyl mandelate **103** in 99% ee and (R)-acetoxy methyl mandelate **104** in 98% ee, **Scheme 78**.



Scheme 78.

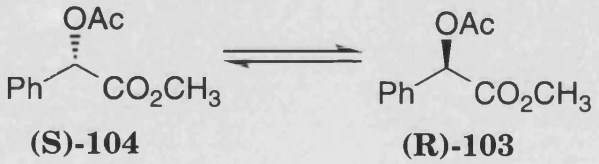
This is the first *kinetic resolution* of acetoxy methyl mandelate to be carried out in an aqueous buffered system and with greater selectivity than the resolutions described by Bevinakatti and Besavaiah.



Scheme 79.

The enzymatic acetylation of methyl mandelate was also successful, **Scheme 79**, yielding both (S)-acetate **104** and (R)-alcohol **103** in excellent ee and conversion.

Racemisation of (S)-acetoxy methyl mandelate was achieved in either organic or aqueous systems, **Scheme 80.**



Conditions	Time days	(OAc)[a] ee %
Bu_4NOAc sat DCM (0.8 ml) 80 °C	3	6
Bu_4NOAc (0.2M, 0.8 ml) / Cs(OAc) 55 °C	2	29

Racemisation experiements were carried out using commercially available (S)-methyl mandelate (99% ee). a) Final enantiomeric excess determined by HPLC analysis.

Scheme 80.

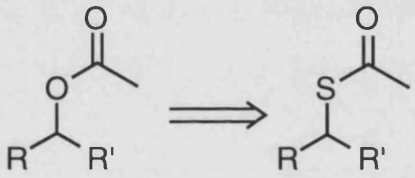
Attempts made at combining the racemisation with the enzymatic hydrolysis were unsuccessful and yielded *kinetic resolution* products, i.e. chiral starting material and product at 50% conversion. This implied that the racemisation was taking place at a slower rate than the enzymatic hydrolysis producing a build up of the 'wrong' enantiomer of substrate that the enzyme was not able to process resulting in a breakdown of the *dynamic resolution*.

4.0 Studies Towards the *Dynamic Resolution* of Thioacetates.

4.1 Introduction.

In the previous Chapters studies were directed towards the *dynamic resolution* of acetates. It was shown in Chapter 3 that the racemisation of acetoxy methyl mandelate was not compatible with an enzyme mediated hydrolysis. The racemisation in this example was taking place at a rate much slower than the rate of hydrolysis. In order to overcome this it was decided to alter the hydrolysed group, i.e. the acetate, into a moiety that could also be hydrolysed by an enzyme and be more susceptible to the continual S_N2 racemisation mechanism.

In order to achieve this the acetate group was substituted for a thioacetate, **Scheme 81**.

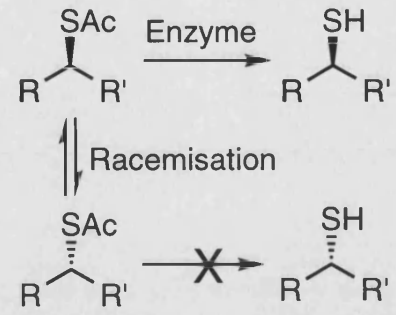


Scheme 81.

It was envisaged that a thioacetate group would be a more suitable stereocentre for this type of racemisation as sulphur is a soft nucleophile and is not as readily solvated compared to an acetate group.

The overall mechanism for the *dynamic resolution* of thioacetates to produce enantiomerically pure thiols is shown below, **Scheme 82**.

Thiols are very important chiral building blocks found in antihypertensive agents and Leukotrienes and there is very little literature precedent for this type of enzymatic conversion, though there are a few papers of interest.

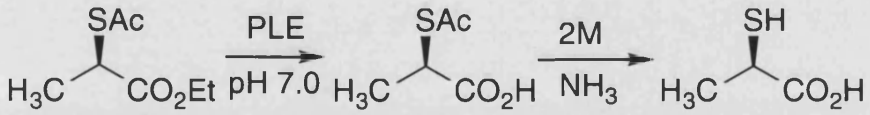


Scheme 82.

Hof and Kellogg [44] demonstrated the use of pig liver esterase (PLE) as a chemoselective catalyst in the hydrolysis of ethyl-2-(acetylsulfanyl)propionate **105**, **Scheme 83**.

In this paper the preparation of both enantiomerically pure (R)- and (S)-thiolactic acids are prepared from a cheap and readily available starting material, (S)-lactate.

At neutral pH PLE hydrolyses selectively the ethyl ester without any racemisation. From experiments using PPL or lipases from *pseudomonas sp.* as catalysts it was noted that thioacetate groups rather than ester groups are usually hydrolysed preferentially, but in the case of using PLE the opposite is true. The thioacetate group will only hydrolyse once all the ester function has been processed.

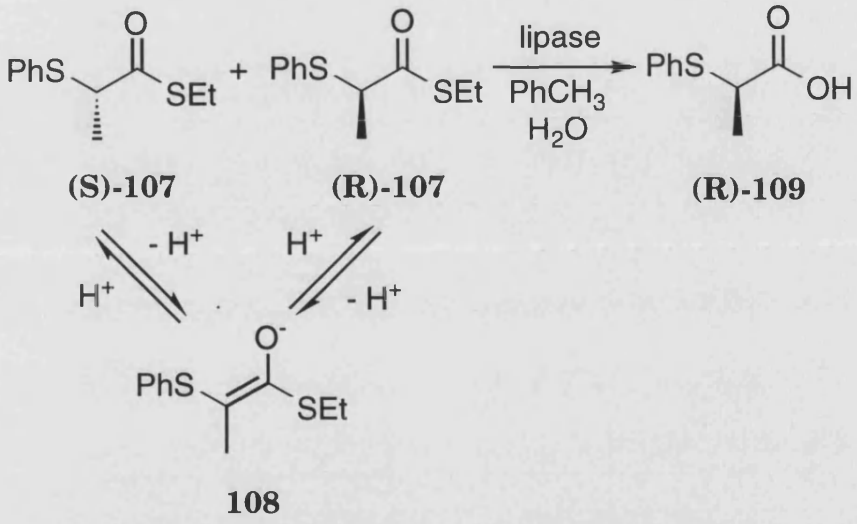


105 **106** **107**

Scheme 83.

The other enantiomer of thiolactic acid, i.e. (S)-thiolactic acid was achieved using the same technique but by treating the opposite enantiomer of starting material **105** to the enzymatic hydrolysis.

Drueckhammer *et al* [45] demonstrated the *dynamic resolution* of a thioester by utilising an enzymatic hydrolysis combined with a base promoted racemisation, **Scheme 84**.



Scheme 84.

In this example the resolution of racemic **107** was initially carried out under non-racemising conditions in a two phase toluene / aqueous system. The pH of the reaction was followed and maintained at pH 7.0 by the addition of 0.2M NaOH.

The authors found that PS-30 (*Pseudomonas cepacia*) resolved the substrate in 96% enantiomeric excess at 19% conversion after 48 hours.

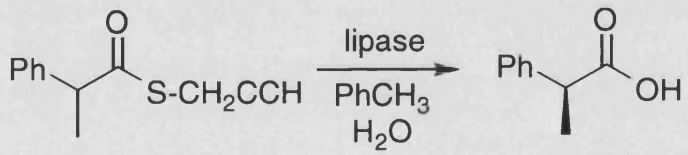
In order to achieve the *dynamic resolution* the substrate was racemised by a base catalysed mechanism. The acidity of the α-proton was increased by the presence of the thioester (compared to the oxoester) and the addition of 0.5 equivalents of trioctylamine resulted in the *in situ* racemisation allowing the final acid product **109** to be isolated in 96% enantiomeric excess at 99% conversion over 65 hours using lipase PS-30.

In a more recent paper Um and Drueckhammer [46] extended this methodology to more substrates and conducted detailed

investigations into the relative rates of racemisation of a series of thioacetates.

In the example below, **Scheme 85** the thioester **110** was subjected to the base catalysed *in situ* racemisation to afford the acid **(S)-109** in very good enantiomeric excess and yield using the lipase Subtilisin Carlsberg as catalyst.

Conducting the hydrolysis without the presence of trioctylamine (nonracemising conditions) produced the (R)-acid **109** in 74% enantiomeric excess at 43% conversion. With the addition of 0.5 equivalents of trioctylamine the substrate was successfully racemised *in situ* to afford **(R)-109** in 80% enantiomeric excess at 95% conversion.

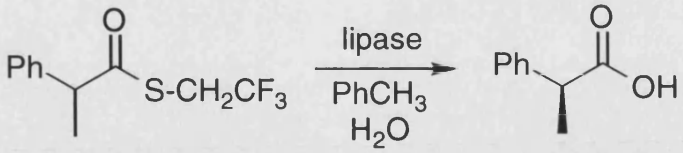


110 **(R)-109**

Conditions	Conv. %	(R)-OH ee %
nonracemising	43	74
racemising	95	80

Scheme 85.

Comparable results were obtained using the trifluoroethyl thioester **111**, **Scheme 86**. In this example nonracemising conditions yielded the acid **(R)-109** in 73% enantiomeric excess at 35% conversion, but with the addition of trioctylamine (racemising conditions) the acid is obtained in 83% enantiomeric excess at 97% conversion.

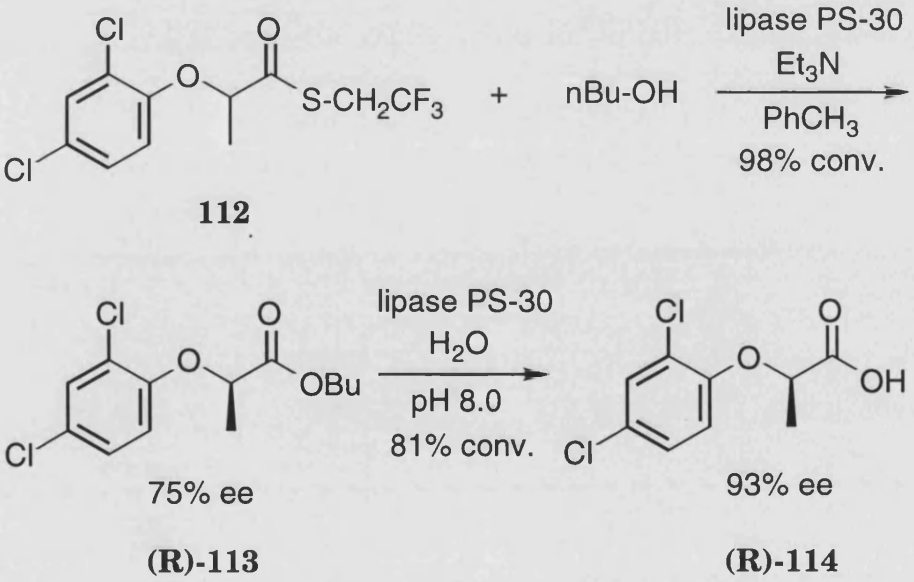


111 **(R)-109**

Conditions	Conv. %	(R)-OH ee %
nonracemising	35	73
racemising	97	83

Scheme 86.

During the study of the enzymatic hydrolysis of **112**, **Scheme 87**, it was noted that substantial non-enzymatic hydrolysis occurred under the racemisation conditions. A transesterification reaction was performed using n-butyl alcohol as the acyl acceptor in the presence of triethylamine, **Scheme 87**. The (R)-butyl ester **113** was obtained in 75% enantiomeric excess at 98% conversion. Hydrolysis of this enantiomerically enriched butyl ester using the same enzyme under nonracemising conditions gave the (R)-acid **114** in 93% enantiomeric excess when the reaction was halted at 81% conversion.



112

75% ee

(R)-113

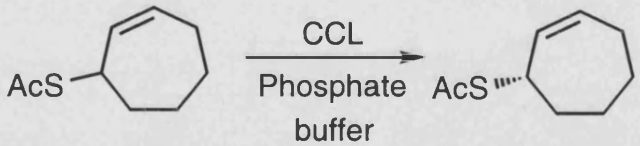
93% ee

(R)-114

Scheme 87.

These examples of enzyme mediated *dynamic resolutions* of thioesters demonstrate one approach to the problem of *in situ* racemisation but is only applicable to substrates with α-protons which are acidic enough to undergo this type of base catalysed mechanism.

Iriuchijima and Kojima [47] in a very early paper demonstrated the first application of hydrolytic enzymes to the hydrolysis of thioacetates. Acetylthiocycloheptene **115**, **Scheme 88**, was treated with CCL in a phosphate buffer and the thioacetate was recovered in 18% yield in 80% enantiomeric excess.



115 **115**

Scheme 88.

It was noted that the thioacetate **115** was hydrolysed more selectively than the corresponding acetate.

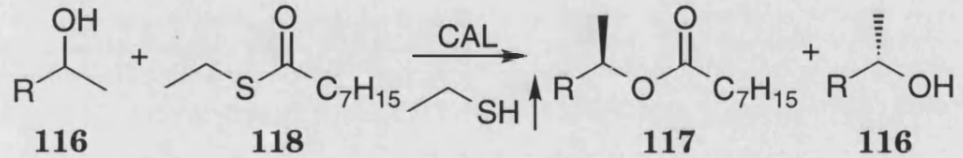
Unfortunately the authors did not isolate or analyse the thiol produced by the enzymatic hydrolysis

An interesting example in the use of a thiooctanoate **118** as acyl donor in the lipase catalysed resolution of a series of secondary alcohols was published by Hult *et al* , [48] **Scheme 89**.

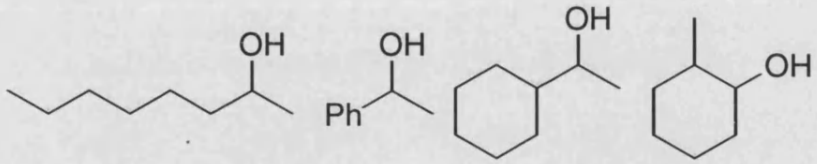
It was shown that by using S-ethyl thiooctanoate **118** the equilibrium of the reaction was pushed more favourably to the products, which were produced with higher enantiomeric excess compared to using ethyl octanoate as acyl donor. The alcohols **116a-d**, were resolved using immobilised CAL in S-ethyl thiooctanoate.

The thioester offered several advantages when used as an acyl donor in the transesterification of alcohols. The co-product formed, ethanethiol, has a low boiling point and was easily removed from the

reaction by evaporation, thus driving the reaction towards the desired ester products resulting in high reaction rates and yields. The thioester was a much better substrate than the produced esters so racemisation of the products was avoided.



116 **118** **117** **116**



a **b** **c** **d**

Substrate	Time hrs	Conv. %	(OR) ee %	(OH) ee %
116a	0.9	52	97	98
116b	2.5	51	97	98
116c	4.4	52	95	98
116d	3.1	50	97	97

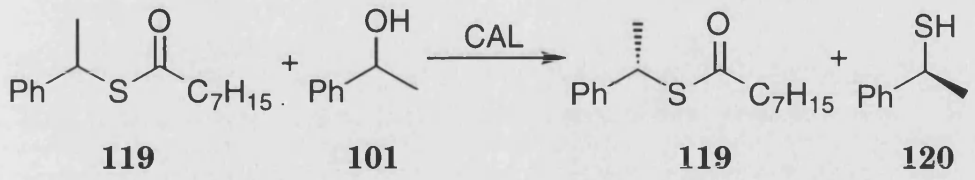
Scheme 89.

All four of the substrates were resolved in very good enantiomeric excess and all substrates were resolved within 4.5 hours highlighting the usefulness of a thioester as an acyl donor in enzymatic transesterification reactions.

As an extension of this in a more recent paper from his group Hult and co-workers [49] resolved the thiooctanoate of phenethyl alcohol **119** using CAL and a corresponding alcohol as an acyl acceptor, **Scheme 90**.

In this example the thiol **120** was isolated in 75% enantiomeric excess at 42% conversion and the remaining thiooctanoate **119** isolated in 95% enantiomeric excess.

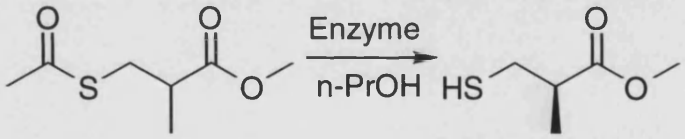
If the hydrolysis was attempted in an aqueous system then the thiol produced was isolated in very poor enantiomeric excess.



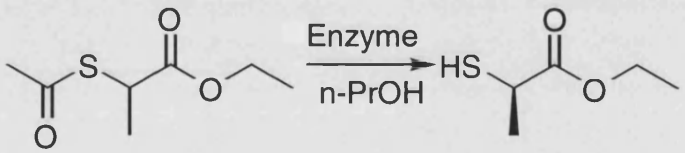
119 **101** **119** **120**

Scheme 90.

Bianchi and Cesti [50] investigated the lipase catalysed hydrolysis of two thioesters **121**, **123**, **Scheme 91**, in an aqueous system but also found that the enantioselectivity of the enzyme suffered. If the reactions were carried out using n-propanol as the nucleophile in an organic medium instead of an aqueous system then enantioselectivity increased dramatically.



121 **122**



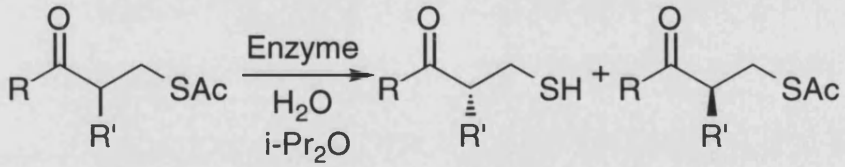
123 **124**

Substrate	Enzyme	Time hrs.	Conv. %	(SH) ee %
121	PCL	24	60	54
121	PPL	72	51	88
123	PCL	32	55	45

Scheme 91.

It was shown that substrate **121** was hydrolysed to the thiol **122** using PPL in 88% enantiomeric excess at 51% conversion using n-propanol. Neither thioacetates **121**, or **123** were hydrolysed using PCL to give the corresponding thiols **122**, **124** in high enantiomeric excesses using n-propanol as the nucleophile but were still substantially better than carrying out the hydrolysis in an aqueous system.

Izawa, Terao, and Suzuki [51] synthesised a series of γ-ketothiols **126** and esters **125** via lipase catalysed hydrolysis in an isopropyl ether / aqueous system, **Scheme 92.**



125 **126** **125**

R	R'	Enzyme	Time hrs.	Conv. %	Config.	(SH) ee %
Ph	C_2H_5	PS	32	43	R	99
Ph	C_2H_5	PL	168	34	R	70
Ph	C_2H_5	CCL	168	33	S	21
Ph	CH_3	PS	25	43	-	99
Ph	CH_3	PL	168	41	-	99
Ph	C_3H_7	PS	30	42	R	99
Ph	$CH(CH_3)_2$	PS	288	42	-	99
Ph	CH_2Ph	PL	24	39	R	99

Scheme 92.

It was found that lipases PL (*Alcaligenes sp.*), and PS (*Pseudomonas cepacia*) all resolved the keto-thioacetates with very good selectivity to produce the (R)-thiol. When CCL was employed in the hydrolysis then not only was enantioselectivity poorer but the opposite enantiomer of keto-thioacetate was hydrolysed to yield the (S)-thiol.

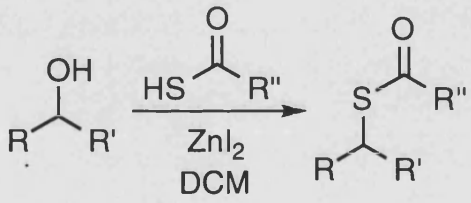
These results encouraged us to try and define the enzymatic hydrolysis of a selection of secondary thioacetates using lipases in an aqueous system.

4.2 Preparation of thioacetates.

A series of easily synthesised secondary thioacetates was required. The substrates had to be synthesised in a few steps from commercially available starting materials.

From the literature thioacetates have been synthesised by many different techniques. These include reaction of thiols with acid chlorides or other activated carboxylic acids, [52] reaction of tosylates or mesylates with thiolacetic acid salts, [53] initial activation of the alcohol with fluoropyridinium salts, [54] or nucleophilic substitution of halides using zinc thioacetates. [55] Unfortunately many of these reactions involved extended purification procedures or low yield of desired thioacetate.

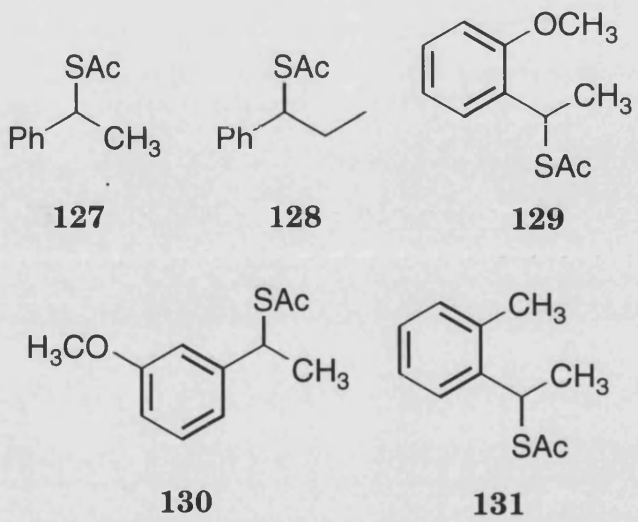
Gauthier *et al* [56] described a direct synthesis of thioacetates from alcohols using thiolacids under Lewis acid catalysis, **Scheme 93**.



Scheme 93.

A secondary alcohol was reacted with either thiolacetic or thiolbenzoic acid using sub-stoichiometric amounts of zinc iodide in dichloromethane to yield the corresponding thioacetate in very good yield without lengthy purification. This was the technique implemented to synthesise the thioacetates **127**, **128**, **129**, **130**, and **131** used in this study, **Scheme 94**.

Phenethyl thioacetate **127**, was synthesised in one step from the commercially available phenethyl alcohol **101** using the methodology described by Gauthier, **Scheme 95**.

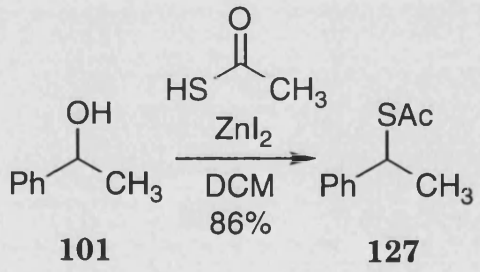


127 **128** **129**

130 **131**

Scheme 94.

Phenethyl alcohol was treated with a slight excess of thiolacetic acid and half an equivalent of zinc iodide in dichloromethane for 4 hours at room temperature. Following an aqueous work up, filtration through a plug of celite, and purification by flash chromatography phenethyl thioacetate **127** was isolated in 86% yield. Appearance of the thioacetoxy methyl singlet at 2.31 ppm in the ^{1}H NMR spectrum indicated the formation of the thioacetate group.

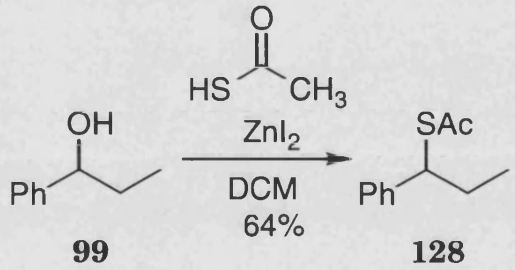


101 **127**

Scheme 95.

Similarly 1-phenyl-1-thioacetoxy propane **128** was synthesised using Gauthier conditions from 1-phenyl propan-1-ol **99**, **Scheme 96**.

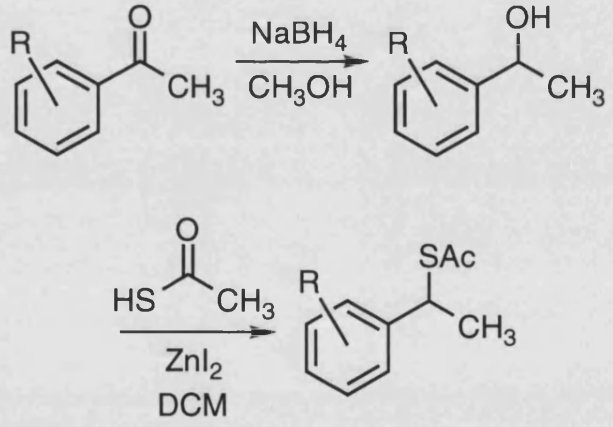
Stirring with thiolacetic acid and zinc iodide in dichloromethane at room temperature for 4 hours, aqueous work up followed by flash chromatography yielded 1-phenyl-1-thioacetoxy-propane, **128** in 64% yield. Again the appearance of the thioacetoxy methyl singlet in the ^{1}H NMR spectrum indicated the formation of the thioacetate.



99 **128**

Scheme 96.

Synthesis of the substituted phenethyl thioacetates **129**, **130**, and **131** followed the general scheme shown below, **Scheme 97**.

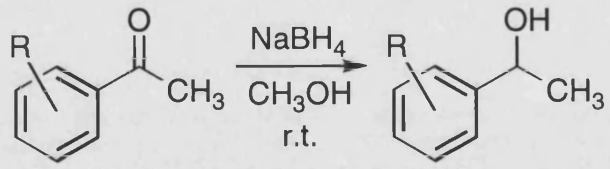


Scheme 97.

Reduction of the ketones **132**, **133**, and **134** with sodium borohydride in methanol, **Scheme 98**, proceeded smoothly to yield secondary alcohols **135**, **136**, and **137** in 96%, 94%, and 94% respectively with no further purification required.

Treatment of alcohols **135**, **136**, and **137** with thiolacetic acid and zinc iodide in dichloromethane at room temperature yielded the desired

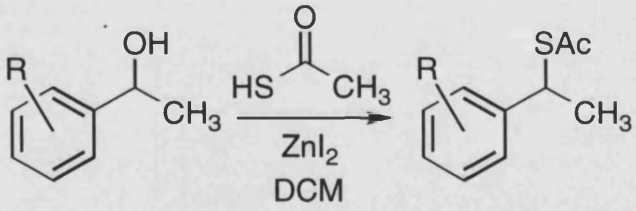
thioacetates, **Scheme 99**. Purification by flash chromatography gave **129**, **130**, and **131** in 87%, 81%, and 63% yield respectively.



132, **135** = o-$(OCH_3)C_6H_4$
133, **136** = m-$(OCH_3)C_6H_4$
134, **137** = o-$(CH_3)C_6H_4$

Scheme 98.

Thioacetate formation was indicated by the appearance of the thioacetoxy methyl singlet in the ^1H NMR spectrum at 2.28, 2.40, and 2.26 ppm respectively for **129**, **130**, and **131**.



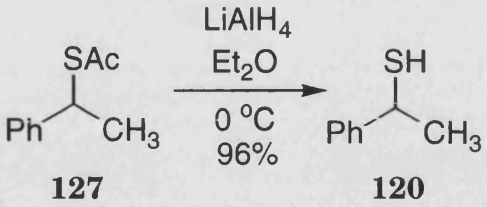
129, **135** = o-$(OCH_3)C_6H_4$
130, **136** = m-$(OCH_3)C_6H_4$
131, **137** = m-$(CH_3)C_6H_4$

Scheme 99.

During the enzymatic hydrolysis of the thioacetates reactions were followed by TLC analysis. A selection of the thioacetates was converted into their corresponding thiols to be used as references.

Hydrolysis of the thioacetates using sodium hydroxide in a water / methanol system was not successful therefore the thioacetates were subjected to hydride reduction using $LiAlH_4$.

Treatment of phenethyl thioacetate **127** in diethyl ether with $LiAlH_4$, **Scheme 100**, produced phenethyl thiol **120** in 96% yield following an acidic work up and flash chromatography.

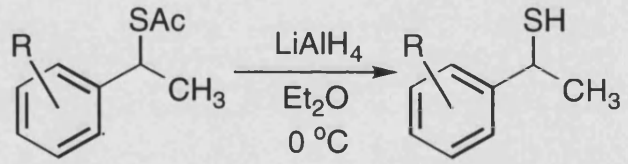


127 **120**

Scheme 100.

Disappearance of the thioacetoxy methyl singlet and appearance of the thiol proton doublet at 1.97 ppm in the ^1H NMR spectrum revealed the formation of the thiol moiety.

Reduction of thioacetates **129**, and **130** with $LiAlH_4$ proceeded very smoothly, **Scheme 101**. Thiols **138**, and **139** were obtained in 95% and 98% yield respectively.



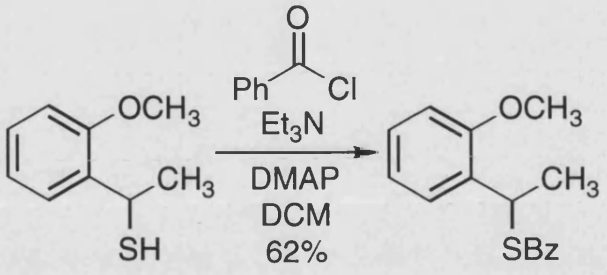
129, **138** = o-$(OCH_3)C_6H_4$
130, **139** = m-$(OCH_3)C_6H_4$

Scheme 101.

Analysis of the thiols produced and the remaining thioacetates was achieved by various HPLC conditions using a Diacel Chirocel OD or OJ column. Where HPLC analysis was not appropriate the products were derivatised and analysed by the addition of a chiral shift reagent or converted into the chiral Moshers ester as outlined below.

4.3 Analysis of o-methoxy phenethyl thioacetate.

o-Methoxy phenethyl thioacetate **129** was derivatised to the thiobenzoate **140**, **Scheme 102**.



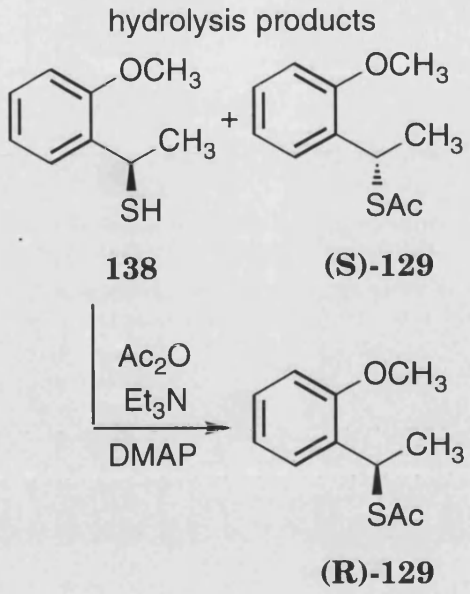
138 **140**

Scheme 102.

Treatment of o-methoxy phenethyl thiol **138** with benzoyl chloride, triethylamine, and a catalytic amount of DMAP yielded o-methoxy phenethyl thiobenzoate **140** in 62% yield.

Unfortunately HPLC analysis did not provide a satisfactory resolution. Addition of chiral NMR shift reagents did resolve the two enantiomers but required an extremely pure sample to be useful. This required extensive purification procedures and was not really a suitable method when many screening experiments were carried out.

Fortunately a useful resolution was obtained by addition of the chiral shift reagent $Eu(hfc)_3$ to a sample of o-methoxy phenethyl thioacetate. Simple re-acetylation of the product thiol, o-methoxy phenethyl thiol **138** to the thioacetate **129** using acetic anhydride, triethylamine, and DMAP, **Scheme 103**, provided an easier route for analysis.

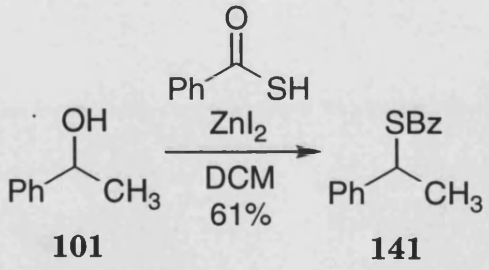


Scheme 103.

4.4 Analysis of phenethyl thioacetate.

A sample of phenethyl thiobenzoate **141** was prepared using Gauthier conditions, **Scheme 104**.

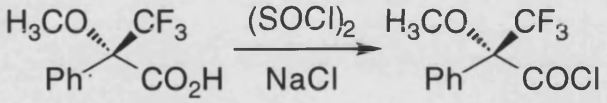
HPLC analysis of phenethyl thiobenzoate was not useful and resolution by the addition of NMR chiral shift reagents produced complicated spectra with no practical application.



Scheme 104

Corey and Cimprich [57] determined the enantiomeric excess of phenethyl thiol by ^{1}H NMR analysis of the corresponding Moshers ester. [58] It was decided to apply this technique to analyse the enantiomeric excess of the reaction products.

(S)-α-methoxy-α-(trifluoromethyl) phenyl acid chloride (S)-MTPACl **143** was prepared from (R)-α-methoxy-α-(trifluoromethyl) phenyl acetic acid (R)-MTPA **142** using the method outlined by Ward and Rhee, [59] **Scheme 105**.



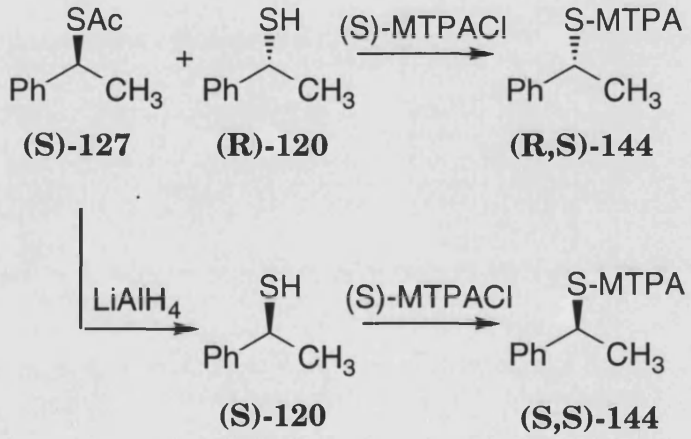
(S)-142 **(R)-143**

Scheme 105.

Separation of the reaction products followed by $LiAlH_4$ reduction of the thioacetate and subsequent conversion to the Moshers ester, **Scheme 106**, provided simple and accurate analysis of the reaction products.

4.5 Enzymatic *Kinetic Resolution* of Thioacetates.

We initially wanted the enzymatic hydrolysis to take place in an aqueous buffered solution. Once this hydrolysis was in place a racemisation protocol would be devised that would be compatible with the enzymatic *kinetic resolution*.



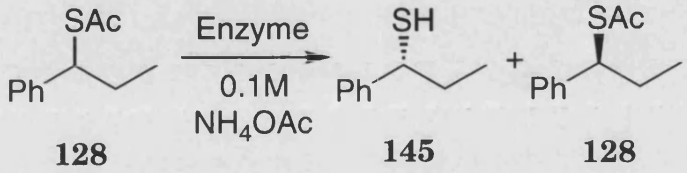
(S)-127 **(R)-120** **(R,S)-144**

(S)-120 **(S,S)-144**

Scheme 106.

Initial experiments were carried out on 1-phenyl-1-thioacetoxy propane **128** screened against our bank of enzymes.

1-Phenyl-1-thioacetoxy propane **128** was screened with each enzyme in ammonium acetate buffer, **Scheme 107**.

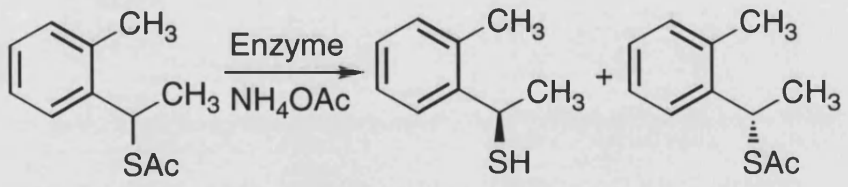


128 **145** **128**

Scheme 107.

No hydrolysis was seen using any of our enzymes and TLC analysis of the extracted products revealed that only starting material was present along with a large amount of baseline material. Flash chromatography isolated starting material (~31%) and the baseline material (~56%) as a foul viscous orange / brown oil that was not identified.

Next o-methyl phenethyl thioacetate **131** was screened with the enzymes, **Scheme 108**.

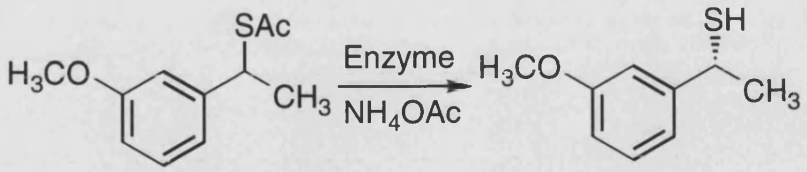


131 **146** **131**

Scheme 108.

Following TLC analysis of the reactions once again no hydrolysis was seen with any of the enzymes used. A large amount of polar baseline material was present in varying amounts and no further purification or identification was attempted.

Not to be discouraged by these initial results we next screened m-methoxy phenethyl thioacetate **130** against our enzymes, **Scheme 109**.



130 **139**

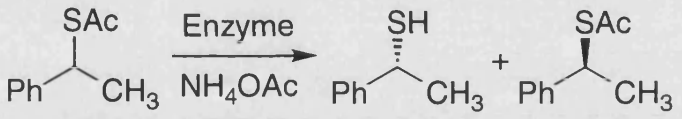
Enzyme	Time days	Conv. %	(SAc) ee %	(SH) ee %
HLE	3	9	40	80
CCL	3	17	30	32
CRL	3	14	14	37

Scheme 109.

Hog liver esterase (HLE) and the lipases isolated from *Candida sp.* (CCL, CRL) all catalysed the hydrolysis to some extent. Unfortunately extensive decomposition was observed in all examples. Here we also see that remaining m-methoxy phenethyl thioacetate **130** was isolated in up to 40% enantiomeric excess at a very low conversion, typically around 9-17%. One explanation for this observation is that the conversion is in fact a lot higher but the thiol formed is reacting further to produce decomposition products resulting in misleading conversions and enantiomeric excess values.

We were not discouraged by these initial results and screened phenethyl thioacetate **127** against our series of enzymes in the ammonium acetate buffer, **Scheme 110**.

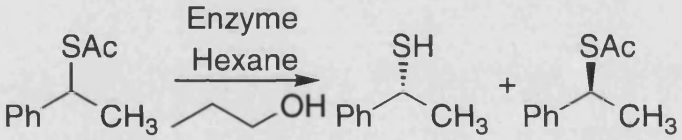
Yet again a large amount of baseline decomposition product was observed with all enzymes screened. Convinced that this must be due to interaction between the reaction products and the buffer a different system was sought.



127 **120** **127**

Scheme 110.

The acyl transfer conditions described by Bianchi and Cesti was put to work on our substrate. [50] Here instead of using water as the nucleophile, n-propanol was used in an acyl transfer reaction, **Scheme 111**.



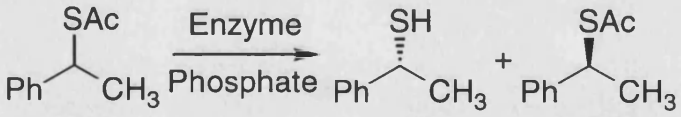
127 **120** **127**

Scheme 111.

We were extremely pleased to see that with all the enzymes screened no decomposition products were present by TLC analysis. We were not so pleased to find that no phenethyl thiol **120** was produced in any of the reactions and an alternative reaction medium was required.

A pot of potassium dihydrogen phosphate was discovered in the chemical store and made up into a 1M solution with water and adjusted to pH 7.0 by the addition of 1M NaOH solution. Phenethyl thioacetate **127** was then screened against our enzymes in this buffer, **Scheme 112**.

The enzymatic hydrolysis of phenethyl thioacetate **127** in phosphate buffer was successfully catalysed by both CCL and HLE.



127 **120** **127**

Enzyme	Time	Temp.	Conv.	(SH)	(SAc)
	days	°C	%	ee %/Config.[a]	ee %/Config.[a]
CCL	4	50	22	25 / (S)	34 / (R)
HLE	7	50	39	57 / (R)	44 / (S)

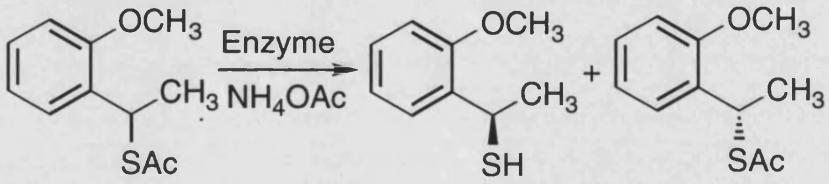
a) Enantiomeric excess and stereochemical configuration determined by ^{1}H NMR analysis of the corresponding Moshers ester and comparison to a sample of phenethyl thiol of known configuration.

Scheme 112.

These are optimised results and demonstrate that HLE catalysed the *kinetic resolution* of phenethyl thioacetate **127** to produce (R)-phenethyl thiol **120** in 57% enantiomeric excess at 39% conversion over 7 days. The remaining (S)-thioacetate was recovered in 44% enantiomeric excess. CCL resolved (R)-phenethyl thioacetate **127** in 34% enantiomeric excess and (S)-thiol **120** in 25% enantiomeric excess at 22% conversion.

During the investigation into the *kinetic resolution* of phenethyl thioacetate we also studied the enzymatic hydrolysis of o-methoxy phenethyl thioacetate **129**.

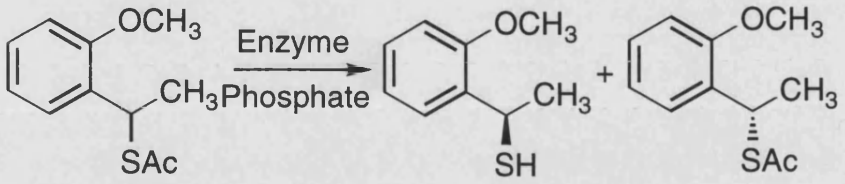
Screening carried out in ammonium acetate buffer, **Scheme 113**, was not too successful and large amounts of decomposition products were isolated from the reactions.



129 **138** **129**

Scheme 113.

Enzymatic acyl transfer reactions carried out in Bianchis' hexane / n-propanol system also failed and o-methoxy phenethyl thioacetate **129** was screened in the phosphate buffer. The hydrolysis was catalysed by CCL and HLE, **Scheme 114**.



129 **138** **129**

Enzyme	Time days	Temp. °C	Conv. %	(SH) ee %	(SAc) ee %
CCL	4	23	25	29	23
HLE	4	23	32	47	48
HLE	7	50	37	54	46

Scheme 114.

The CCL catalysed hydrolysis resolved o-methoxy phenethyl thioacetate **129** in 23% enantiomeric excess and o-methoxy phenethyl thiol **138** in 29% enantiomeric excess in 25% yield over 4 days. HLE hydrolysed the opposite enantiomer to produce o-methoxy phenethyl thiol **138** in 54% enantiomeric excess in 37% yield and o-methoxy phenethyl thioacetate **129** in 46% enantiomeric excess.

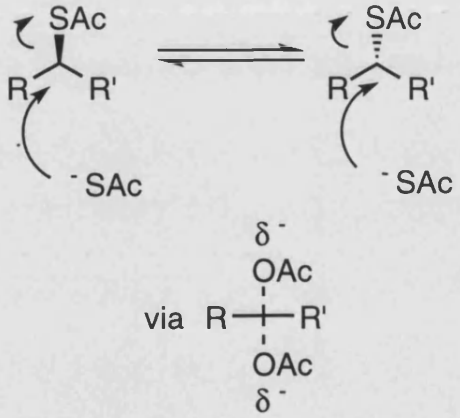
Pleased with these results we next investigated a suitable racemisation protocol to combine with the enzymatic *kinetic resolution* of each thioacetate to effect the *dynamic resolution*.

4.6 Racemisation of phenethyl thioacetate.

As demonstrated in Chapter 3 our strategy was to achieve racemisation via a series of S_N2 displacements of the thioacetate by another thioacetate group from the reaction system, **Scheme 115**.

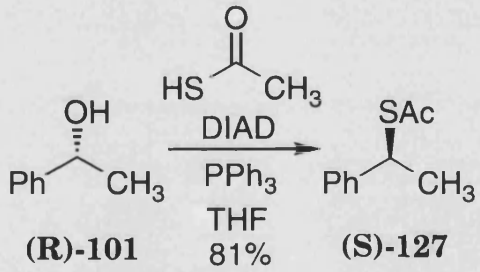
Racemisation experiments required an enantiomerically pure sample of phenethyl thioacetate **127** that was synthesised by treatment of (R)-phenethyl alcohol **101** with Mitsonobu inversion conditions, [60] **Scheme 116**.

Due to the extended analysis time required for determination of enantiomeric excesses an easily analysed test system was devised to explore racemisation conditions.



Scheme 115.

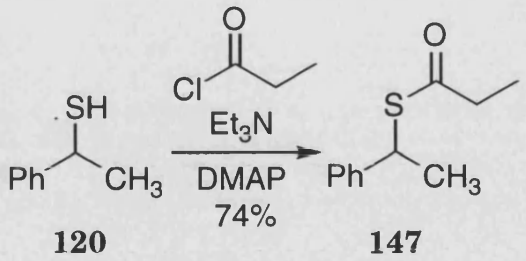
The ethyl thioester **147** was easily synthesised from phenethyl thiol **120** in one step by treatment with propanoyl chloride, **Scheme 117**.



Scheme 116

It was predicted that if this ethyl thioester was treated with a thioacetate source then S_N2 displacement would occur to yield an amount of phenethyl thioacetate. Both phenethyl thioethanoate **147** and phenethyl thioacetate **127** were easily separated by GC analysis

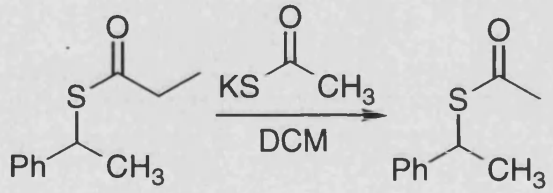
using a fused silica column and allowed easy analysis of reaction products.



120 **147**

Scheme 117.

Initial studies were carried out using commercially available potassium thioacetate as a thioacetate source, **Scheme 118**.



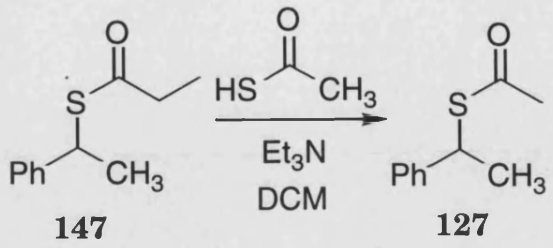
147 **127**

Conditions	Temp °C	Time hrs.	Yield[a] (SCOEt)	Yield[a] (SAc)
$KSCOCH_3$ (10 eq) / DCM	23	4	98	0
$KSCOCH_3$ (5 eq) / DCM	50	18	95	2

a) Yields determined by GC analysis of reaction mixture.

Scheme 118.

Unfortunately no useful amount of displacement was observed with this system and more forcing conditions, using thiolacetic acid and triethylamine were examined, **Scheme 119**.



147 **127**

Conditions	Temp °C	Time hrs.	Yield[a] (SCOEt)	Yield[a] (SAc)
$HSCOCH_3$ (0.5 eq) Et_3N (0.5 eq) / DCM	50	48	93	4
$HSCOCH_3$ (5 eq) Et_3N (5 eq) / DCM	23	18	0	96

a) Yields determined by GC analysis of reaction mixture.

Scheme 119.

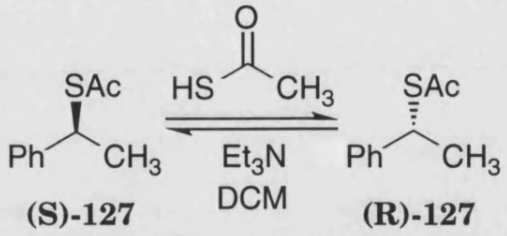
No displacement occurred using 0.5 equivalents of thiolacetic acid and triethylamine but complete conversion was observed with 5 equivalents of thiolacetic acid and triethylamine in dichloromethane. These reactions were carried out in sealed tubes.

It was now known that a large excess of thiolacetic acid was able to displace the ethylthioester and these conditions were applied to the racemisation of phenethyl thioacetate, **Scheme 120**.

After 1 day the enantiomeric excess had only fallen by ~9% at 50 °C. If the reaction was allowed to run for longer than after 4 days a decrease in enantiomeric excess was noted, 99% to 68%. Allowing the reaction to run for 7 days resulted in phenethyl thioacetate **127** being isolated in 53% enantiomeric excess at 34% yield.

4.7 Conclusions.

In this Chapter we demonstrated that the enzymatic *kinetic resolution* of thioacetates was not as straightforward as their oxoacetate cousins. The results presented in this Chapter are the result of many months worth of blood, sweat, and towards the final stages, tears but the results were worth the effort.



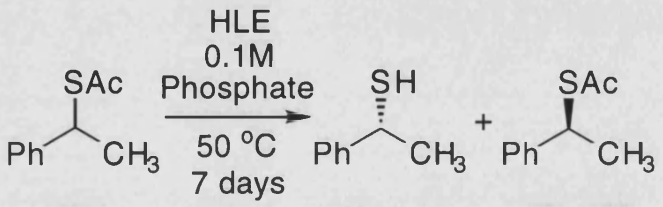
(S)-127 **(R)-127**

Conditions (SAc)	Temp.	Time	Yield
	°C	days	%	ee %
$HSCOCH_3$ (5 eq) / Et_3N (5 eq) / DCM	50	1	32	91
$HSCOCH_3$ (4 eq) / Et_3N (4 eq) / DCM	40	4	29	68
$HSCOCH_3$ (5 eq) / Et_3N (5 eq) / DCM	40	7	34	53

Scheme 120.

All thioacetates screened underwent substantial decomposition if the lipase catalysed hydrolysis was carried out in an acetate rich environment, i.e. ammonium acetate buffer.

Phenethyl thioacetate **127** was resolved by HLE in a phosphate buffer system at 50 °C over 7 days to yield (S)-phenethyl thioacetate **127** in 57% enantiomeric excess and (R)-phenethyl thiol **120** in 44% enantiomeric excess at 39% conversion, **Scheme 121**.

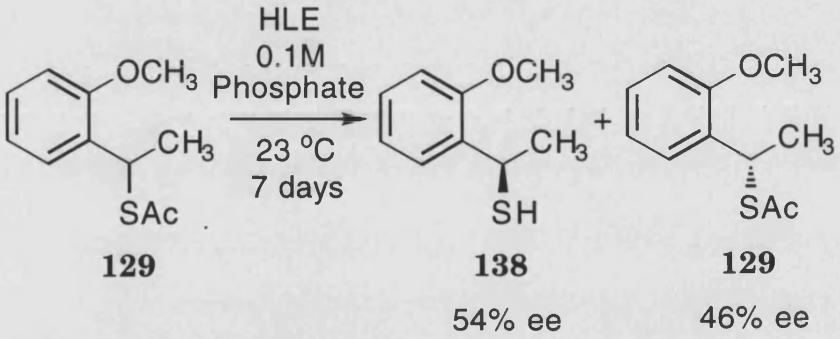


127 **(R)-120** **(S)-127**

57% ee 44% ee

Scheme 121.

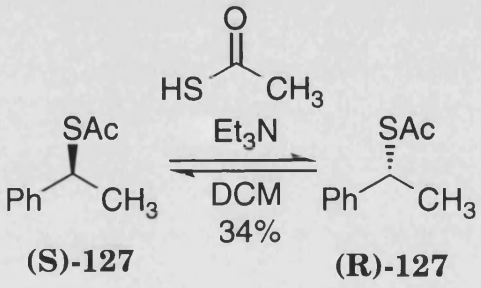
Similarly o-methoxy phenethyl thioacetate **129** was resolved by HLE in the phosphate buffer at 23 °C over 7 days to yield o-methoxy phenethyl thioacetate **129** in 46% enantiomeric excess and o-methoxy phenethyl thiol **138** in 54% enantiomeric excess at 37% conversion, **Scheme 122**.



Scheme 122.

These results are amongst the first examples of lipase catalysed *kinetic resolutions* of thioacetates to be carried out in an aqueous buffered solution without using an organic acyl transfer system.

Racemisation of phenethyl thioacetate **127** was successfully accomplished by treatment of an enantiomerically pure sample of (S)-phenethyl thioacetate with 5 equivalents of thiolacetic acid and triethylamine in DCM over 7 days at 40 °C, **Scheme 123**.



Scheme 123.

Due to experimental complications the two systems were not combined to afford a *dynamic resolution* though I feel that with the recent commercial availability of crosslinked enzyme crystals (CLEC enzymes, see next Chapter) and their ability to tolerate much harsher reaction conditions a *dynamic resolution* of thioacetates is awaiting the next brave chemist with a poor sense of smell and high powered fume hoods.

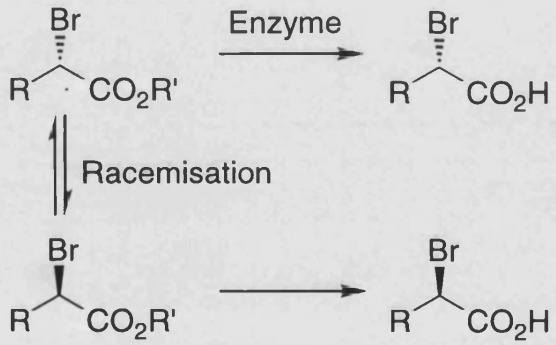
5.0 Studies Towards the *Dynamic Resolution* of α-Bromo Esters.

5.1 Introduction.

The previous Chapter had concentrated upon the hydrolysis of acetoxy and thioacetoxy containing substrates. Racemisation was achieved via a series of S_N2 displacements of the thioacetoxy group.

In this Chapter a slightly different approach to the continuous S_N2 *in situ* racemisation combined with an enzymatic hydrolysis will be described.

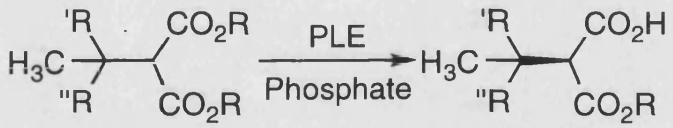
Our strategy is outlined below, **Scheme 124**. An α-bromo ester will be subjected to an enzymatic *kinetic resolution*, converting the ester into the corresponding carboxylic acid. In the presence of a bromide source an *in situ* S_N2 racemisation would be carried out allowing *dynamic resolution* of the α-bromo ester.



Scheme 124.

Schafer *et al* [61] investigated the Pig Liver esterase (PLE) catalysed selective hydrolysis of a series of tertiary alkyl diesters, **Scheme 125**. Here a series of diacetates **148** was treated with PLE in a phosphate buffer system.

In all cases the monoesters **149** were isolated in very good yields and enantiomeric excesses.



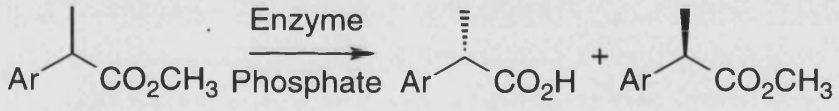
148 **149**

R'	R"	R	Conv. %	Yield %	(CO_2H) ee %
CH_3	CH_3	CH_3	100	87	89
CH_3	CH_3	C_2H_5	100	86	92
CH_3	C_2H_5	C_2H_5	90	75	82
CH_3	Ph	C_2H_5	90	70	94
CH_3	Ph	CH_3	80	68	90

Scheme 125.

Here we see that hydrolytic enzymes are capable of performing regio-selective hydrolysis of diesters.

Gu, Chen, and Sih [62] utilised an enzymatic *kinetic resolution* in the enantiomeric synthesis of (S)-2-(6-methoxy-2-naphthyl) propionic acid, Naproxen, **Scheme 126.**



150 **151** **150**

Ar = p-(OCH_3)-Naphyl

Enzyme	Conv. %	Ester ee %	Acid ee % / Config.
CCL	39	63	> 98 / S
M. meihei	18	21	95 / R
R. arrhizus	11	13	97 / R
Rhizopus sp.	19	21	92 / R
R. oryzae	11	10	76 / R

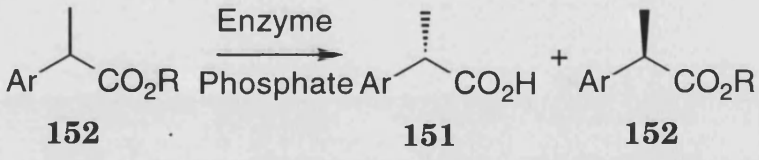
Scheme 126.

2-Arylpropionic acids are an important class of non-steroidal anti inflammatory drugs, two of the most commonly prescribed are p-isobutylhydratropic acid (Motrin), and (S)-2-(6-methoxy-2-napthyl) propionic acid (Naproxen) **151**.

The authors had previously noted that *Penicillium vinaceum* and *Streptomyces cavourensis* catalysed this hydrolysis to yield the acid **151** in very high enantiomeric excess (98% ee). Unfortunately the rate of hydrolysis was very slow and these enzymes had a very low substrate concentration tolerance (< 5 g/l) and so this process was not practical for large scale application.

In this study the authors discovered that lipases from *Rhizopus, Mucor,* and *Candida* were able to catalyse the hydrolysis of the methyl ester **150** to the corresponding carboxylic acid **151**. These lipases were more stable and were able to tolerate higher concentrations of substrate (> 1M). It was shown that all of the enzymes screened preferentially hydrolysed the undesired (R) enantiomer and only CCL successfully cleaved the desired (S) enantiomer.

It was also shown that the enantio selectivity of CCL was not affected by the ester function. This lead to the use of activated esters in the enzymatic *kinetic resolution* of **152, Scheme 127**.



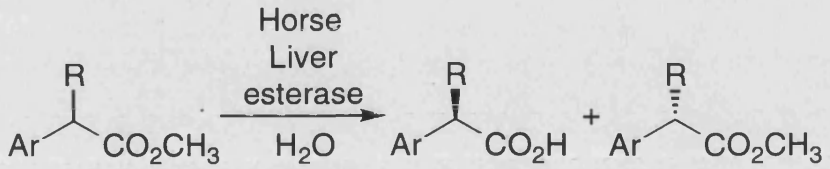
152 **151** **152**

Ar = p-(OCH_3)Naphthyl

R	Relative Rate
CH_3	1
CH_2CH_2Cl	15
CH_2CN	6
$CH_2CH(NO_2)CH_3$	3

Scheme 127.

Ahmar, Girard, and Bloch [63] described the enzymatic *kinetic resolution* of 2-alkyl-2-aryl acetic methyl esters **153**, **Scheme 128**, using Horse Liver esterase in water.



153 **154** **153**

a : Ar=Ph, R=CH_3
b : Ar=p-(OCH_3)Ph, R=CH_3
c : Ar=m-(OCH_3)Ph, R=CH_3
d : Ar=o-(OCH_3)Ph, R=CH_3
e : Ar=p-(i-Bu)Ph, R=CH_3 (Ibuprofen)

Substrate	Time hrs	Conv. %	(acid) ee %	(ester) ee %
a	1.75	40	92	48
b	1	42	91	43
c	1.75	45	62	46
d	2.5	41	67	40
e	11	40	88	60

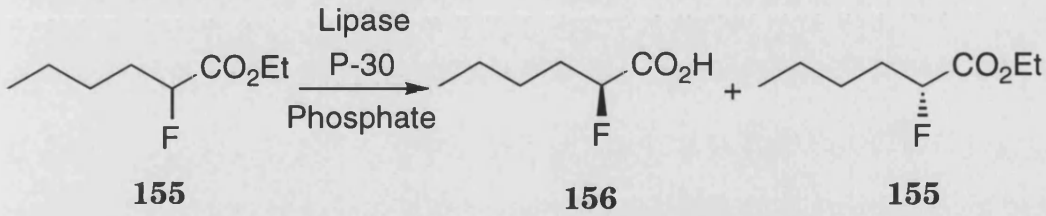
Scheme 128.

It was shown that all the methyl esters **153a-e** screened were good substrates for Horse Liver esterase catalysed hydrolysis. In all cases the (R)-enantiomer was selectively hydrolysed to yield the corresponding (R)-carboxylic acid **154a-e** in moderate to good enantiomeric excesses.

The authors also noted that Pig Liver esterase (PLE) hydrolysed very quickly substrate **153a** but unfortunately the hydrolysis was not selective and racemic acid was isolated.

These examples display the practical use of hydrolytic lipases in the enantiomeric synthesis of carboxylic acids using very mild conditions and are often carried out in aqueous systems.

Kalaritis and Regenye [64] utilised Lipase P-30 to selectively hydrolyse ethyl 2-fluorohexanoate **155**, **Scheme 129**. The reaction was carried out in a phosphate buffer maintained at pH 7.0 by the continual addition of 0.1M NaOH.

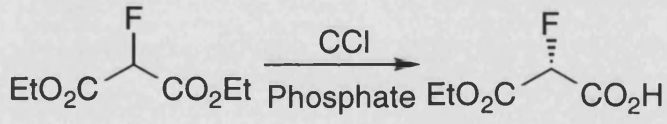


155 **156** **155**

Scheme 129.

In this example the α-fluoro ethyl ester **155** was selectively hydrolysed to the (S)-α-fluoroacid **156** in 86% enantiomeric excess using Lipase P-30 in a phosphate buffer if the conversion was stopped at 40%. Re-esterification of this acid **156** and once again treating this with the enzymatic hydrolysis conditions yielded α-fluoroacid **156** in 99% enantiomeric excess.

Kobyashi *et al* [65] utilised CCL in the desymmetrisation of a series of α-fluoro diesters , **Scheme 130**.



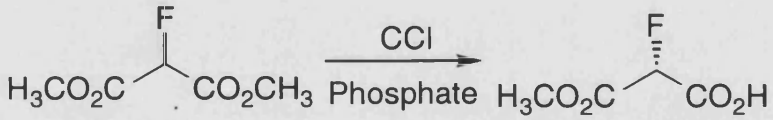
157 **158**

Scheme 130.

Here the α-fluoro diester **157** was treated with CCL in a phosphate buffer while maintaining the reaction at pH 7.3 to yield the α-fluoro monoester **158** in 94% enantiomeric excess in 91% yield. In this

example the (R)-enantiomer was produced. When the α-fluoro diester **157** was hydrolysed using *Trichoderma viride* cellulase the (R)-enantiomer was produced in lower yield (73%) and enantiomeric excess (38%).

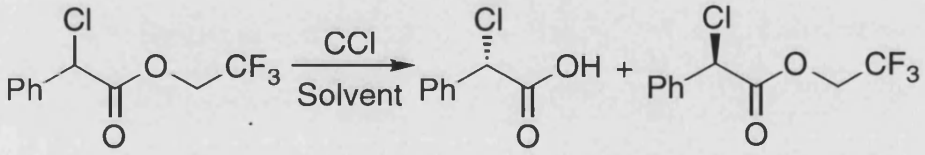
The methyl-α-fluorodiester **159**, **Scheme 131**, was also treated with CCL to produce the (R)-monoester **160** in 91% enantiomeric excess in 87% yield. *Trichoderma viride* cellulase hydrolysed the diester **159** in a reduced selectivity to produce (R)-monoester **160** in 56% enantiomeric excess at 42% yield.



159 **160**

Scheme 131.

Garcia *et al* [66] utilised CCL in the enzymatic *kinetic resolution* of 2,2,2-triflouroethyl-α-chloro-α-phenyl acetate **161**, **Scheme 132**.

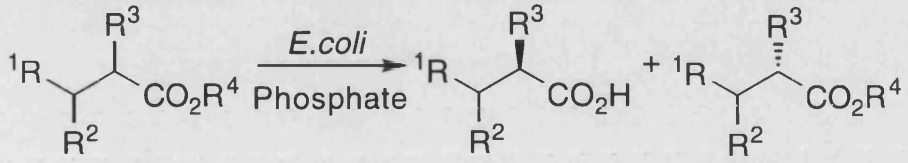


161 **162** **161**

Scheme 132.

Here it was demonstrated that 2,2,2-trifluoroethyl-α-chloro-α-phenyl acetate **161** was hydrolysed to (S)-α-chloro phenyl acetate **162** in 94% enantiomeric excess at 46% conversion when the hydrolysis was carried out in 0.1M phosphate buffer. When the hydrolysis was carried out in hexane with 20% water added to the solution the enantiomeric excess of (S)-α-chloro phenyl acetate **162** fell to 70% at 39% conversion.

Ozaki and Sakashita [67] studied the effects of a recently isolated enzyme source, *E.coli* C600 / pPE117, [68] on a series of α-substituted carboxylic acid derivatives **163** including the α-chloro substituted methyl ester **163** (R^1 = OH, R^2 = H, R^3 = Cl) , **Scheme 133**.



163 **164** **163**

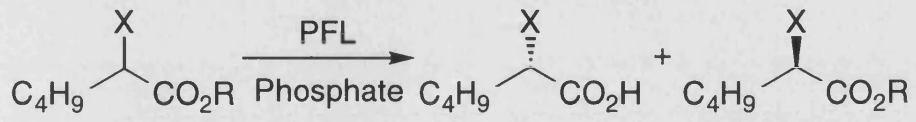
R^1	R^2	R^3	R^4	Conv. %	(ester) ee %	(acid) ee %
SAc	H	CH_3	CH_3	50	98	99
OCH_3	H	CH_3	CH_3	50	98	98
OH	H	Cl	CH_3	77	98	30

Scheme 133.

In this work it was demonstrated that *E.coli* C600 / pPE117 is a highly selective esterase for the hydrolysis of this type of substrate. It is interesting to note that the enzyme selectively hydrolysed the methyl ester in the presence of a thioacetoxy substituent (R^1= SAc, **163**).

Here it was also shown that when the α-chloro methyl ester (R^2 = Cl, **163**) was hydrolysed using *E.coli* C600 / pPE117 the α-chloro carboxylic acid (R^2 = Cl, **164**) was isolated in 30% enantiomeric excess at 77% conversion. The authors postulate that the substrate was subject to chemical hydrolysis in the buffered solution. If this was the case then the starting material recovered from the reaction would also display reduced enantiomeric excess, but here the methyl ester (R^2 = Cl, **163**) was recovered in 98% enantiomeric excess. A more suitable explanation for this observed enantiomeric enrichment of the recovered starting material is that *E.coli* C600 / pPE117 is not selective to the produced α-chloro carboxylic acid.

Kalaritis *et al* [69] studied the enzymatic hydrolysis of a series of α-substituted hexanoates **165** using *Pseudomonas fluorescens* lipase (PFL) in a 0.05M aqueous phosphate buffer, **Scheme 134**.



165 **166** **165**

X	R	Conv. %	(R)-(ester) ee %	(S)-(acid) ee %
F	C_2H_5	50	84	-
F	C_2H_5	60	99	69
Br	CH_3	60	94	93
Br	n-Bu	50	72	-
Br	C_2H_5	50	73	70

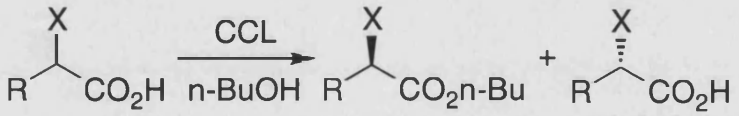
Scheme 134.

All the substrates studied were hydrolysed preferentially to the (S)-enantiomer by PFL, which is the opposite to the expected (R)-selectivity usually displayed by this lipase. [70]

The α-fluoro ethyl ester (X = F, R= Et, **165**) was hydrolysed to the (S)-acid (X = F, **166**) in 69% enantiomeric excess at 60% conversion. Allowing the conversion to progress above 50% enabled the remaining (R)-α-flouro ethyl ester (X = F, R = Et, **165**) to be isolated in 99% enantiomeric excess.

Enzymatic hydrolysis of the α-bromo methyl ester (X = Br, R = Me, **165**) yielded the (S)-α-bromo acid (X = Br, **166**) in 93% enantiomeric excess at 60% conversion. The recovered (R)-α-bromo methyl ester (X = Br, R = Me, **165**) was isolated in 94% enantiomeric excess. When this methyl ester was substituted for an ethyl ester (X = Br, R = Et, **165**) then the enantiomeric excess of the (S)-α-bromo acid (X = Br, **166**) falls to 70% at 50% conversion.

Kirchner, Scollar, and Klibanov [71] investigated the CCL catalysed esterification of a series of α-halo carboxylic acids **167**, **Scheme 135** to produce enantiomerically enriched n-butyl esters **168**.



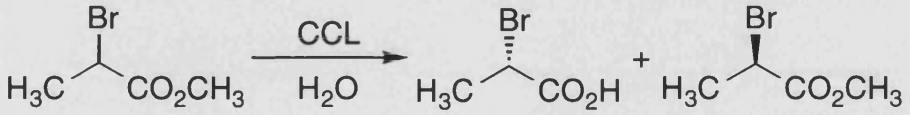
167 **168** **167**

R	X	Time hrs.	Conv %	(R)-(ester) ee %
CH_3	Br	6	45	96
CH_3	Cl	6	42	95
$CH_3(CH_2)_3$	Br	19	30	99
Ph	Cl	168	8	99
Ph	Br	216	20	99

Scheme 135.

All substrates were resolved in very high enantiomeric excesses though the esterification of α-chloro phenyl acetic acid (X = Cl, R = Ph, **167**), and α-bromo phenyl acetic acid (X = Br, R = Ph, **167**) was very slow, 8% conversion after 168 hours for the α-chloro acid (X = Cl, R = Ph, **168**) and 20% conversion after 216 hours for α-bromo phenyl acetic acid (X = Br, R = Ph, **168**).

Dahod [72] utilised CCL in the enzymatic *kinetic resolution* of methyl-α-bromo propionate **169**, **Scheme 136**.



169 **170** **169**

Scheme 136.

Dahod demonstrated that methyl-α-bromo propionate **169** when treated with CCL in water maintained at pH 6.2 for 24 hours was

successfully resolved in 85% enantiomeric excess at 43% conversion. When repeated on a batch scale α-bromo propionic acid **170** was isolated in 73% enantiomeric excess after 96 hours.

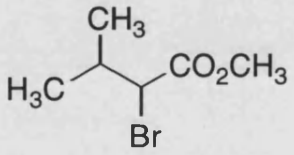
Due to the synthetic importance of enantiomerically pure α-halo carboxylic acids many patents are held describing enzymatic *kinetic resolutions* of this type of substrate. The enzymatic resolution of α-chloro carboxylic esters [73] and α-bromo carboxylic esters [74] being the most patented processes.

With this literature precedent we were optimistic that we would be able to design a *dynamic resolution* methodology for the complete resolution of a series of α-bromo methyl esters.

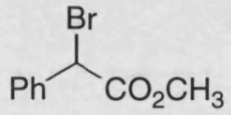
5.2 Preparation of α-bromo esters.

A series of either commercially available or easily synthesised α-bromo esters was required. The substrates used in this study are shown below, **Scheme 137**.

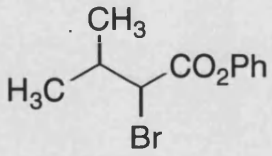
Methyl-α-bromo phenyl acetate **171**, and methyl-α-bromo propionate **169** were purchased directly from commercial sources.



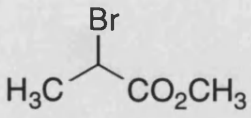
173



171



175

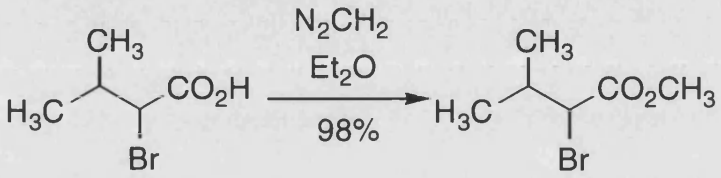


169

Scheme 137.

Methyl-2-bromo-3-methyl butanoate **173** was easily synthesised from commercially available 2-bromo-3-methylbutanoic acid **174**, **Scheme 138**.

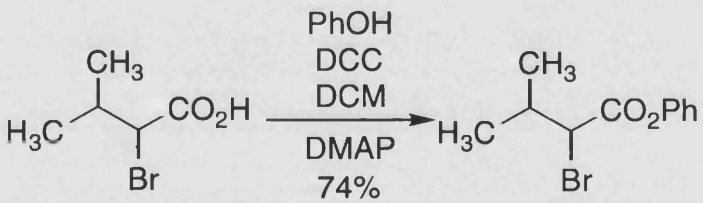
Treatment of the acid **174** in diethyl ether with diazomethane yielded methyl-2-bromo-3-methyl butanoate **173** in 98% yield with no further purification required.



174 **173**

Scheme 138.

Phenyl-2-bromo-3-methyl butanoate **175** was also easily synthesised from 2-bromo-3-methylbutanoic acid **174**, **Scheme 139**.



174 **175**

Scheme 139.

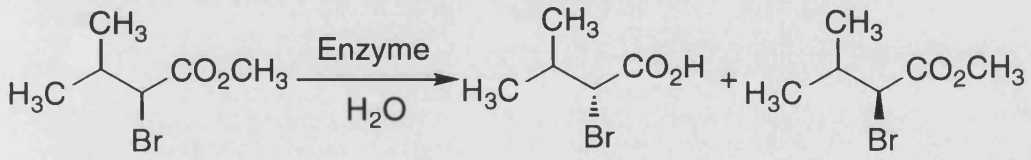
Treatment of the α-bromo carboxylic acid **174** with DCC coupling conditions with phenol in dichloromethane in the presence of a catalytic amount of DMAP produced the phenyl ester. Following filtration through a plug of silica and flash chromatography phenyl-2-bromo-3-methyl butanoate **175** was isolated in 74% yield.

With our series of α-bromo esters in hand we next investigated the enzymatic *kinetic resolution* of these substrates using our bank of enzymes.

5.3 Enzymatic *kinetic resolution* of α-bromo esters.

Initially we envisaged the enzymatic hydrolysis to be carried out in an aqueous environment and combining this with an appropriate racemisation protocol.

Initial work was carried out on methyl-2-bromo-3-methyl butanoate **173**, and this was screened against our enzymes, **Scheme 140**.



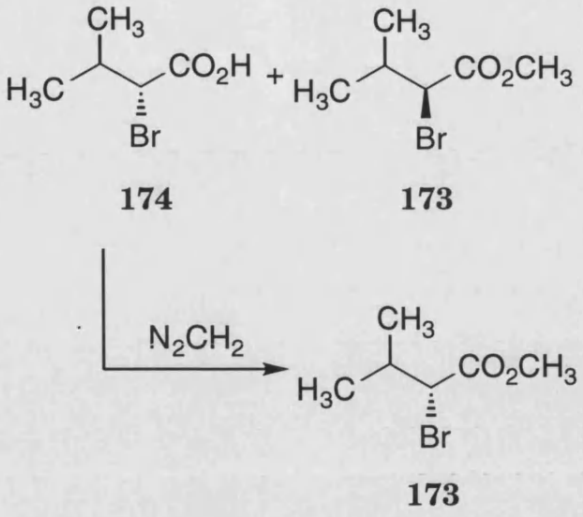
173 **174** **173**

Enzyme	Time	Temp.	Conv.	(acid)[a,b]	(ester)[b]
	hrs	°C	%	ee %	ee %
CCL	18	30	22	47	62
PFL	18	30	11	29	48
HLE	18	30	14	23	10
CRL	18	30	28	39	64
CAL	18	30	9	11	18

Absolute configuration is unknown. a) Acid converted into methyl ester (diazomethane). b) Enantiomeric excess determined by GC analysis (γ-cyclodextrin, 50m, 60 °C).

Scheme 140.

Analysis was carried out by acid, base work up and treatment of the isolated acid **174** with diazomethane to afford the corresponding methyl ester **173** , **Scheme 141**, which was subsequently analysed by capillary GC techniques.



Scheme 141.

It was discovered that all the enzymes screened were active in catalysing the enzymatic hydrolysis of methyl-2-bromo-3-methyl butanoate **173** to 2-bromo-3-methylbutanoic acid **174** but conversions and enantioselectivities varied greatly.

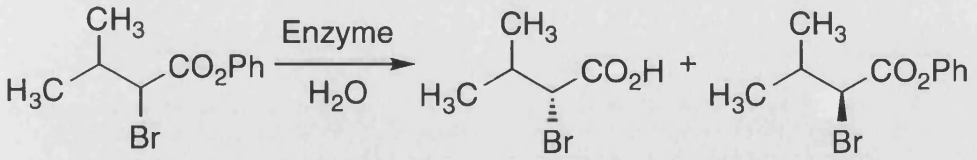
The lipases from *Candida sp.* CCL, and CRL displayed the greatest enantioselectivity, 2-bromo-3-methylbutanoic acid **174** being isolated in 47% and 39% enantiomeric excess at 22% and 28% conversion respectively. Also the *Pseudomonas sp.* lipases PFL and PCL displayed some selectivity, α-bromo acid **174** being isolated in 29% and 24% enantiomeric excess respectively.

It was interesting to note that CCL and CRL hydrolysed the opposite enantiomer to the rest of the enzymes screened here, although configuration remains unknown.

Next phenyl-2-bromo-3-methyl butanoate **175** was screened with our enzymes in water, **Scheme 142**.

Here we see that the α-bromo phenyl ester **175** was hydrolysed at a much slower rate than the α-bromo methyl ester **173**. After stirring in water for 48 hours with 30 mg of each enzyme at pH 7.0 only CCL and PCL displayed any catalytic activity. CCL hydrolysed the α-bromo phenyl ester **175** to produce the α-bromo acid **174** in 4% enantiomeric excess at 19% conversion. The resolution with PCL was

also similar, α-bromo acid **174** was isolated in 22% enantiomeric excess at 12% conversion.



175 **174** **175**

Enzyme	Time	Temp.	Conv.	(acid)[a]	(ester)[b]
	hrs	°C	%	ee %	ee %
CCL	48	30	19	4	6
PCL	48	30	12	22	15
PFL	48	30	10	16	12

a) Acid converted to methyl ester (diazomethane), enantiomeric excess determined by GC analysis (γ-cyclodextrin, 50m, 60 °C) b) Enantiomeric excess determined by GC analysis (γ-cyclodextrin, 50m, 120 °C).

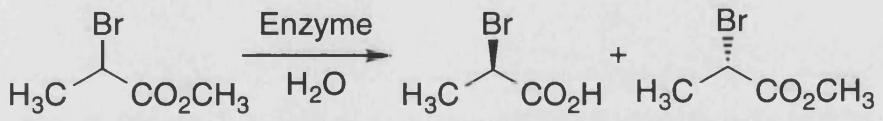
Scheme 142.

The size of the hydrolysed moiety, i.e. the phenolic ester, may account for the extended reaction times and reduced conversions. The steric bulk of the phenyl ring may not allow the substrate to comfortably fit into the enzymes active site, thus hydrolysis is slower and enantiomeric selectivity may suffer as a consequence. The electron withdrawing nature of the phenyl π-electrons may activate the ester towards chemical hydrolysis which would result in the loss of enantiomeric excess.

The enzymatic hydrolysis of methyl-α-bromo propionate **169**, provided more encouraging results, **Scheme 143**.

CCL hydrolysed the α- methyl ester **169** to the α-bromo acid **170** in 59% enantiomeric excess at 46% conversion after 5 hours. CRL catalysed the hydrolysis in 5 hours to form α-bromo acid **170** in 38% enantiomeric excess at 68% conversion. PFL produced α-bromo acid **170** in 22% enantiomeric excess at 39% conversion after 48 hours

while PCL at 50% conversion displayed very little enantioselectivity (3 %) after 5 hours.



169 **170** **169**

Enzyme	Time hrs	Temp. °C	Conv. %	(acid)[a] ee %	(ester)[a] ee %
CCL	5	35	46	59	52
PFL	48	35	39	22	34
HLE	5	35	61	9	19
CRL	5	35	68	38	43
PCL	48	35	50	2	8
CAL	48	35	19	3	6

a) Enantiomeric excess determined by GC analysis (γ-cyclodextrin, 50 m, 34 °C).

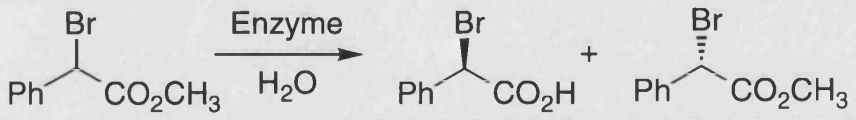
Scheme 143.

Though enantiomers were not distinguished CCL and CRL both hydrolysed the opposite enantiomer to all the other enzymes screened.

These results were encouraging. Though the enantiomeric excess of the α-bromo acid produced were still below 60% they represented a significant improvement to the results already obtained.

Next we decided to replace the alkyl group with a larger phenyl group in an attempt to increase the enantioselectivity displayed by the enzyme. It was hoped that the large aryl terminus would allow better distinction of the substrate at the enzymes active site and shorten analysis times as no derivatisation of the reaction products would be required.

Methyl-α-bromo phenyl acetate **171** was screened with our enzymes in water, **Scheme 144**.



171 **172** **171**

Enzyme	Time hrs.	Temp. °C	Conv. %	(acid)[a] ee % / Config[b]	(ester)[a] ee %
CRL	18	22	42	74 / (S)	69
CCL	18	22	36	68 / (S)	62
CCL	24	40	41	72 / (S)	60
PFL	48	40	19	14 / (R)	18
PCL	48	40	27	31 / (R)	21
CAL	48	40	13	24 / (S)	24
Novo 435	2.5	22	30	10 / (R)	4
Novo 677	24	30	34	27 / (R)	15
Flavourzyme	16	22	9	51 / (R)	12

a) Enantiomeric excess determined by HPLC analysis (Diacel Chiracel OD, 48:2:0.4 n-hexane:IPA:HCOOH), b) Stereochemical configuration determined by comparison of optical rotation to a sample of acid of known stereochemistry.

Scheme 144.

Even from these initial experiments a large improvement in resolution was seen. All the enzymes screened displayed improved enantioselectivity resolving both α-bromo acid **172** and remaining α-bromo methyl ester **171** in modest to good enantiomeric excess.

As before the lipases isolated from *Candida sp.* provided the best resolution. CRL and CCL resolved the (S)-α-bromo acid **172** in 74% and 68% enantiomeric excess respectively at 42% and 36% conversion after 18 hours. Novo Flavourzyme resolved the opposite (R)-enantiomer in 51% enantiomeric excess but conversion was low (9%) and as a consequence the remaining (S)-α-bromo methyl ester **171** was isolated in 12% enantiomeric excess.

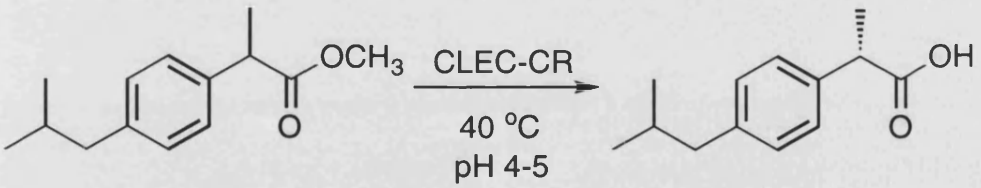
5.4 Cross-linked Enzyme Crystals, CLEC enzymes.

CLEC enzymes can be considered 'purified' enzymes. Most commercially available enzyme preparations contain several other different hydrolases in addition to the lipase major component. These 'rogue' hydrolases may reduce the catalytic activity and / or enantio selectivity displayed by the enzyme towards a specific substrate.

It could be considered that 'rogue' hydrolases present in the lipase could hydrolyse the substrate with no enantioselectivity, or selectively hydrolyse the opposite (R)-enantiomer thus reducing the enantioselectivity of the overall process.

CLEC enzymes are cross linked microcrystals obtained from lipases. The major lipase constituent is isolated and cross linked with glutaraldehyde to produce 'pure' lipase crystals with all other minor hydrolases removed. This type of enzyme preparation display greater stability due to both the crystallinity and the crosslinks, and improved enantioselectivity due to the absence of other hydrolases.

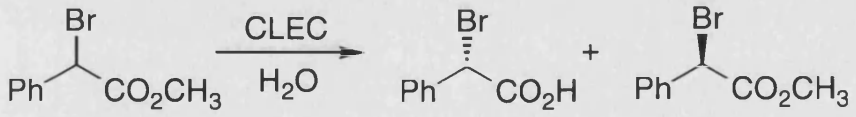
CLECs have found widespread application in the resolution of racemic compounds [75] and have been employed in the *kinetic resolution* of methyl ibuprofen **153e** to produce (S)-ibuprofen **154e** in 93% enantiomeric excess in 38% conversion using CLEC-CR isolated from CRL, [76] **Scheme 145.**



153e **154e**

Scheme 145.

With the recent commercial availability of CLEC enzymes it was decided to apply these preparations to the *kinetic resolution* of methyl-α-bromo phenyl acetate **171** with the intent of improving the enantiomeric excess of the α-bromo acid **172** produced, **Scheme 146**.



171 **172** **171**

Enzyme	Time hrs.	Conv. %	(acid)[d] ee % / Config.[e]	(ester)[d] ee %
CLEC-CR [a]	2.5	47	80 / (S)	81
CLEC-PC [b]	144	32	65 / (R)	51
CLEC-BL [c]	9	15	12 / (R)	19

Reactions carried out at room temp. a) Isolated from *Candida rugosa* lipase. b) Isolated from *Pseudomonas cepacia* lipase. c) Isolated from *Substilisin carlsberg* protease. d) Enantiomeric excess determined by HPLC analysis. e) Confirmed by optical rotation.

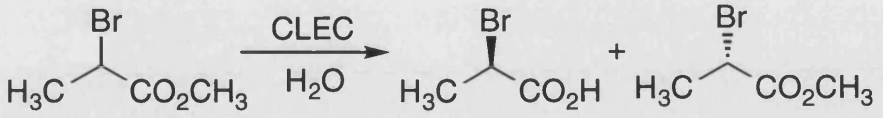
Scheme 146.

We were delighted to see that both CLEC-CR (CRL), and CLEC-PC (*Pseudomonas sp.*) resolved the α-bromo methyl ester **171** in good enantiomeric excess.

CLEC-CR produced (S)-α-bromo acid **172** in 80% enantiomeric excess at 47% conversion, and the remaining (R)-α-bromo methyl ester **171** was isolated in 81% enantiomeric excess. CLEC-PC also catalysed the hydrolysis to give the opposite (R)-α-bromo acid **172** in 65% enantiomeric excess but at a much slower reaction rate. CLEC-BL did not display any selectivity and almost racemic products were obtained from the reaction.

CLEC enzymes were also applied to the *kinetic resolution* of methyl-α-bromo propionate **169**, **Scheme 147**.

The CLEC catalysed enzymatic hydrolysis of methyl-α-bromo propionate **169** did not provide any significant improvement. The CLEC-CR catalysed hydrolysis reached 54% conversion after 1.5 hours, compared with 5 hours for the corresponding reaction using CCL. Enantioselectivity was not improved upon and α-bromo propionoic acid **170** was isolated in similar enantiomeric excess, 64% and 59% for CLEC-CR and CCL respectively.



169 **170** **169**

Enzyme	Time	Conv.	(acid)[a]	(ester)[a]
	hrs.	%	ee %	ee %
CLEC-CR	1.5	54	64	69
CLEC-PC	12	43	26	42
CLEC-BL	12	18	19	7

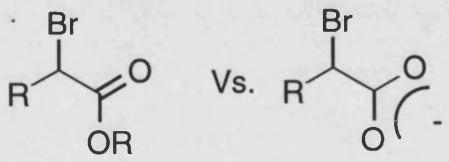
Reactions carried out at room temp. a) Enantiomeric excess determined by HPLC analysis.

Scheme 147.

With time pushing on it was decided to examine and identify a suitable *in situ* racemisation protocol that would be compatible with the CLEC-CR catalysed *kinetic resolutions*.

5.5 Selective racemisation of α-bromo esters.

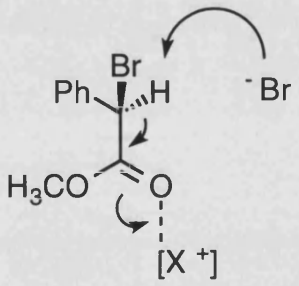
Racemisation of the α-bromo esters was to be achieved by the continual S_N2 mechanism discussed in previous Chapters. In these examples the racemisation was to take place by the continual displacement of the bromide by another bromide from the reaction system. Due to the chemical differences of the substrate ester and product acid only the starting material would be racemised by an S_N2 mechanism, **Scheme 148**.



Scheme 148.

During racemisation the incoming bromide displaces the α-bromide of the ester substrate. The transient species formed involves the formation of a tight transition state where the bond formed and the breaking bond have proceeded to the same extent and this is stabilised by the donation of electron density into the π^* orbital of the carbonyl carbon. Once the ester has been hydrolysed to the carboxylic acid then the situation has changed. The enzymatic hydrolysis is carried out at neutral pH and the product acid exists as the carboxylate shown. Now the S_N2 mechanism is greatly retarded. The π^* orbitals are now filled by the electron density being transferred during the resonance of the carboxylate, preventing the displacement of the bromide by a second bromide resulting in no further racemisation.

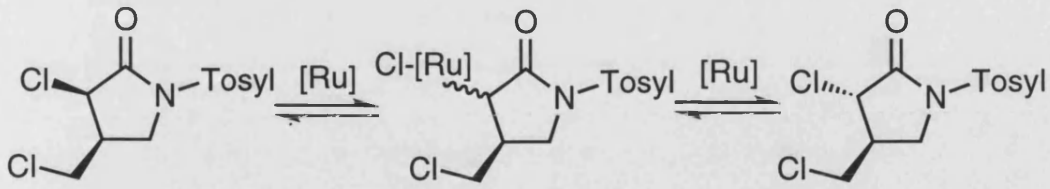
An alternative Lewis acid catalysed racemisation has not been ruled out, **Scheme 149**. Here the positively charged bromide counter ion acts as a Lewis acid and promotes deprotonation resulting in racemisation of the substrate. All racemisation reactions were carried out at neutral pH to limit any enolisation taking place. Deuterium exchange experiments are required to rule out this mechanism.



Scheme 149.

We aimed to carry out the racemisation of the α-bromo methyl ester in an aqueous system using a labile bromide source that would not interfere with the enzymatic process taking place.

Stereochemical scrambling of α-halo compounds has a literature precedent. Slough [77] studied the $(Ph_3P)_3RuCl_2$ catalysed equilibration of a series of N-tosyl-2-pyrrolidinones, **Scheme 150**.

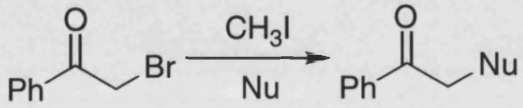


[Ru]= $(Ph_3P)_3RuCl_2$

Scheme 150.

Slough demonstrated that epimerisation at this centre occurred by an α-chloride atom abstraction and return mechanism mediated by the ruthenium complex.

Halvorsen and Songstad [78] studied the reactivity of 2-bromo-1-phenylethanone **176** towards nucleophilic attack by a variety of nucleophiles including other halogens, **Scheme 151**.

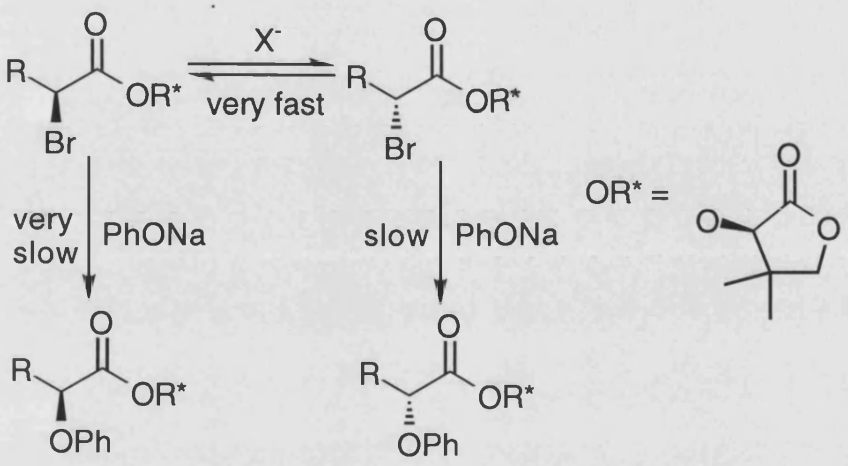


176 **177**

Scheme 151.

In this work the authors discovered that α-carbonyl groups are important for increasing the rate of S_N2 displacement with nucleophiles that can form tight transition states. The charge and polarisability of the nucleophile is more important for nucleophiles that are unable to form this tight transition state.

Koh and Durst [79] utilised a quaternary ammonium iodide salt to racemise an α-bromo carboxylic ester. The authors utilised this racemisation to synthesise a series of (S)-aryloxy acids, **scheme 152**.



Scheme 152.

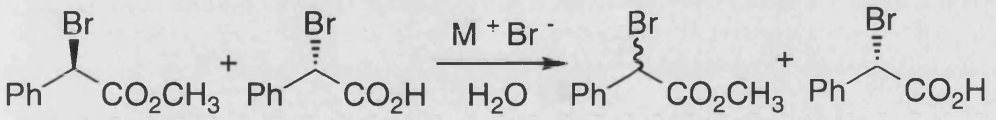
Here (S)-aryloxy esters were obtained in diasterioselectivities up to 95:5. This is due to epimerisation of the slower reacting (S)-α-halo ester into the faster reacting (R)-α-halo ester by the halide generated in the displacement reaction or from halide added to the reaction system in the form of a quaternary ammonium halide salt.

This observation was also utilised by Caddick and Jenkins as outlined in Chapter 1.

Our initial experiments were carried out on enantiomerically enriched methyl-α-bromo phenyl acetate **171**. in the presence of enantiomerically enriched α-bromo phenyl acetic acid **172**. Racemisation was to be achieved by using a sub-stoichiometric amount of a quaternary ammonium bromide salt as a bromide source, **Scheme 153**.

It was refreshing to see something work on the first attempt. Selective racemisation of methyl α-bromo phenyl acetate **171** in the presence of α-bromo phenyl acetic acid **172** was achieved by most of the quaternary salts screened.

Good results were obtained with tetrabutylphosphonium bromide, racemising the ester **171** in 18 hours at room temperature, hexadecyltriphenylphosphonium bromide effecting racemisation in 6 hours. Tetraoctylammonium bromide racemisised the methyl ester at room temperature along with hexadecyl pyridinium bromide.



(R)-171 **(S)-172** **+/- 171** **(S)-172**

X^+Br^-	Time	(ester)[a]		(acid)[a]	
	hrs.	init ee %	final ee %	init ee %	final ee %
Bu_4PBr	18	81	4	33	31
$C_{16}H_{21}PPh_3Br$	6	55	5	38	36
CsBr	18	80	77	35	35
KBr	18	38	28	34	34
$BnPPh_3Br$	2	82	40	74	69
$(C_8H_{17})_4NBr$	72	30	6	56	52
$C_{10}H_{21}PyBr$	18	31	4	60	51
$Me_3N(CH_2)_{10}$-NMe_3 2 Br^-	18	66	59	79	79
$(CH_3)_4N$ $3Br^-$	18	82	74	74	72
Bu_4NBr	18	81	80	78	78
$(CH_3)_4NBr$	18	57	54	42	42

Reactions carried out at room temp., neutral pH, < 0.6 equivalents of bromide salt used in all experiments. a) Enantiomeric excess determined by HPLC analysis.

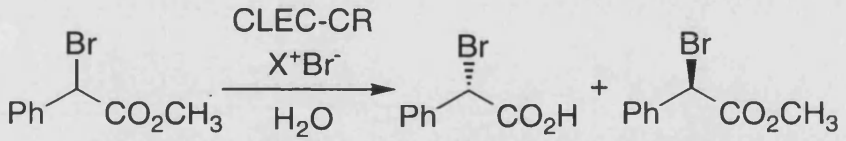
Scheme 153.

It was intersting to note that 'simple' bromide salts, i.e. CsBr and KBr did not effect racemisation of the methyl ester **171**. This may be caused by the relative solvation of the cation. The larger cation is easily solvated and so releases more 'free' bromide into the solution.

5.6 *Dynamic resolution* of methyl-α--bromo phenyl acetate.

With the racemisation now in place it only remained to combine the enzymatic *kinetic resolution* with this racemisation. Hexadecyltriphenylphosphonium bromide and tetrabutylammonium bromide were combined with the CLEC enzymes, **Scheme 154**.

It was found that tetrabutyl phosphonium bromide did not afford a quick enough racemisation and there was a build up of the unwanted isomer resulting. (R)-α-bromo phenyl acetic acid **172** was isolated in 68% enantiomeric excess at 56% conversion. The remaining (S)-methyl-α-bromo phenyl acetate **171** was isolated in 34% enantiomeric excess indicating that the racemisation was occurring at a slower rate than the hydrolysis.



171 **172** **171**

CLEC	X^+Br^-	Time hrs.	Conv. %	(acid)[a] ee % / Yield %	(ester)[a] ee %
CR	$C_{16}H_{21}PPh_3Br$	7	70	68 / 65	6
PC	Bu_4PBr	144	56	68 / 51	34

a) Enantiomeric excess dertermined by HPLC analysis.

Scheme 154.

Hexadecyltriphenylphosphonium bromide was able to racemise the substrate at a rate fast enough to ensure that a continual supply of the correct enantiomer of methyl ester **171** (6% ee) was available. A *dynamic resolution* of methyl-α-bromo-phenyl-acetate **171** was achieved. The (S)-α-bromo acid **172** was isolated in 68% enantiomeric excess in 65% overall yield after 7 hours at room temperature.

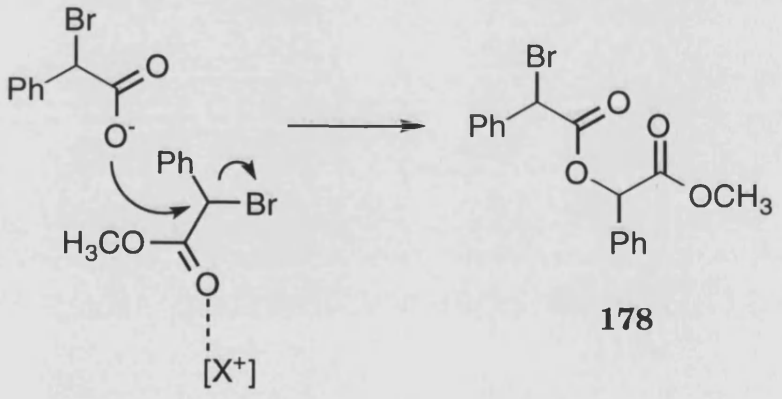
It was found that at conversions above 70% a second product was being formed by the reaction conditions. Isolation of this compound was not easy. The r.f. value was very close to that of the original α-

bromo methyl ester **171**. Isolation was acheived by simple acid base work up and prep. TLC methods to yield a pale yellow oil.

During racemisation the bromide counter ion could could act as a Lewis acid, thus promoting the continual bromide S_N2 displacement mechanism.

We postulated that at a sufficient concentration of α-bromo acid **172** this carboxylate could displace the bromide atom on methyl-α-bromo phenyl acetate **171**. This effect could be catalysed by the bromide source cation, **Scheme 155**.

^1H and ^{13}C NMR analysis revealed the identity of the 'mystery' compound to be the bromide substituted compound **178**. This evidence was backed up by accurate mass analysis displaying the molecular ion, M^+ at 362.0162.

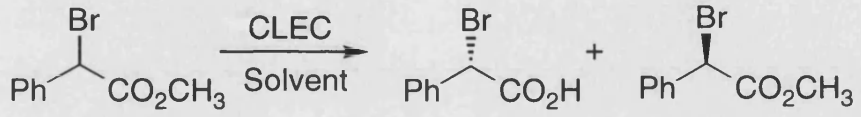


178

Scheme 155.

While this was an intersting observation this side product was adversely effecting the overall efficiency of the *dynamic resolution*. In order to reduce this displacement reaction we investigated the use of organic additives to the reaction system. We re-screened methyl-α-bromo phenyl acetate **171** with CLEC-PC and CLEC-CR in the two component solvent systems, **Scheme 156**.

These are unoptimised results but demonstrate that CLEC-CR was capable of resolving the α-bromo methyl ester **171** with good enantioselectivity.



171 **172** **171**

Enzyme	Solvent	Time hrs.	Conv. %	(acid)[a] ee % / Config	(ester)[a] ee %
CLEC-PC	12.5:1 H_2O/DCM	18	34	62 / (R)	34
CLEC-PC	10:1 H_2O/EtOH	8	57	4 / (R)	45
CLEC-CR	5:1 H_2O/MeOH	5	32	61 / (S)	55
CLEC-CR	5:1 H_2O/MeCN	4	24	63 / (S)	57
CLEC-CR	5:1 H_2O/n-hexane	5	17	43 / (R)	51

a) Enantiomeric excess determined by HPLC analysis.

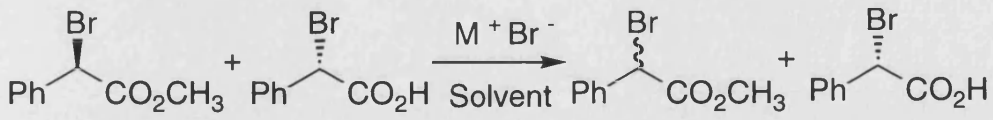
Scheme 156.

The bromide sources were then re-screened in the two phase systems, **Scheme 157**.

All of the systems investigated racemised methyl-α-bromo phenyl acetate **171** to some extent. It was unfortunate to note that α-bromo phenyl acetic acid **171** was also being racemised by these conditions, enantiomeric excess of the recovered α-bromo acid **171** was depleted by up to 40%.

Next the above systems were combined to achieve the *dynamic resolution*, **Scheme 158**.

It was disappointing to find that the *dynamic resolution* was not taking place. The two phase water / hexane system did not afford a quick enough racemisation and a build up of the wrong (R)-enantiomer of α-bromo methyl ester **171** was seen. Homogenious water and methanol conditions produced α-bromo phenyl acetic acid **172** in poorer enantioselectivity with no racemisation of the starting material.



(R)-171 **(S)-172** **+/- 171** **(S)-172**

X^+Br^-	Solvent	Time	(ester)[a]		(acid)[a]	
		hrs.	init. ee%	final ee%	init ee%	final ee%
Bu_4NBr	H_2O/Et_2O	96	60	3	59	39
Bu_4NBr	Et_2O	18	79	4	67	51
Bu_4NBr	H_2O/MeOH	4	81	1	64	35
Bu_4NBr	H_2O/MeCN	18	80	2	63	41
Bu_4PBr	Et_2O	18	69	46	61	52
Bu_4PBr	H_2O/MeOH	3	33	1	37	27
$C_{16}H_{21}$-PPh_3NBr	H_2O/MeCN	4	78	4	72	32

a) Enantiomeric excess determined by HPLC analysis.

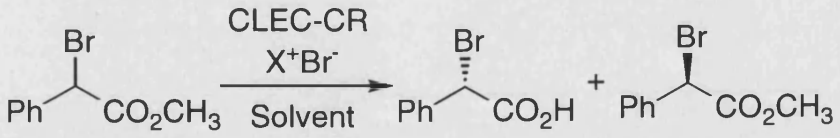
Scheme 157.

It was obvious that this type of system was not going to work for us. Though no side reaction was taking place the racemisation and enzymatic hydrolysis conditions were not working hand in hand and an alternative strategy was considered.

5.7 Polymer bound phosphonium bromide salts.

It was thought that the cationic species of the bromide source was catalysing the side reaction. If we could remove this from the reaction then complete conversion would be obtained without any side reaction taking place.

Hughes [80] investigated the use of polymer bound quaternary phophonium bromides as a traceless linker for synthesis of small organic molecules. Hughes utilised the polymer bound phosphonium **179** as an immobilised Wittig reagent [81] in the synthesis of stilbene **180**, **Scheme 159**.



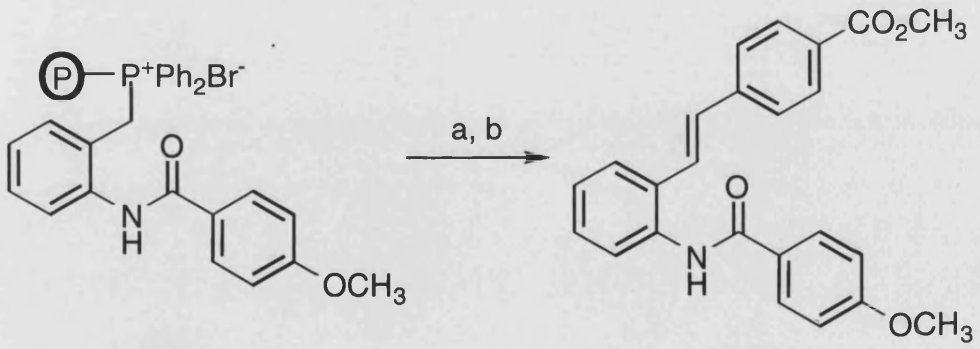
171 **(S)-172** **(R)-171**

Conditions	Time hrs.	Conv. %	(acid)[a] ee % / Config	(ester)[a] ee %
CLEC-CR/Bu_4NBr/ 5:1 H_2O/n-hexane	10	42	72 / (S)	79
CLEC-CR/Bu_4PBr/ 5:1 H_2O/n-hexane	5	24	71 / (S)	43
CLEC-CR/Bu_4PBr/ 5:1 H_2O/MeOH	6	29	22 / (S)	26
CLEC-CR/$C_{10}H_{21}PPh_3Br$/ 5:1 H_2O/MeOH	6	47	65 / (S)	54

a) Enantiomeric excess determined by HPLC analysis.

Scheme 158.

It was envisaged that a solid polymer bound quaternary phosphonium bromide salt would act as a bromide source and effect the racemisation of methyl-α-bromo phenyl acetate in an aqueous medium.



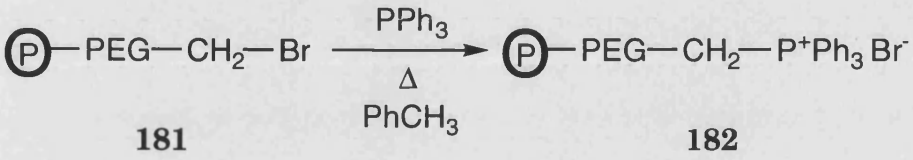
179 **180**

a) Methyl-4-formylbenzoate (2 eq), NaOMe, MeOH, reflux, 2 hrs. b) Girards Reagent T (3 eq), AcOH, 18 hrs.

Scheme 159.

Two different polymer supported phosphonium bromide salts were synthesised from commercially available brominated resins.

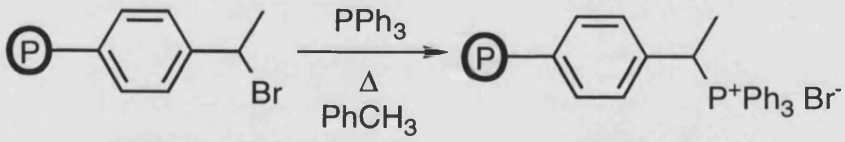
Brominated Tentagel (TG) **181** was quaternised with excess triphenylphosphine in toluene to form the quaternised phosphonium salt **182, Scheme 160**.



181 **182**

Scheme 160.

Similarly brominated Wang resin **183** was quaternised using triphenyl phosphine in toluene under reflux to form the immobilised phosphonium salt **184, Scheme 161**.



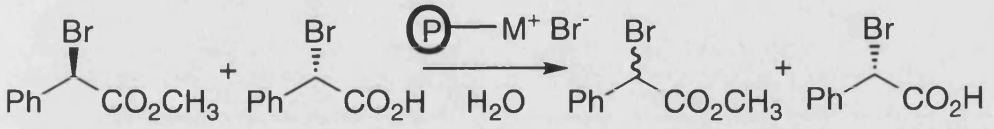
183 **184**

Scheme 161.

Enantiomerically enriched methyl-α-bromo phenyl acetate **171** and α-bromo phenyl acetic acid **172** were then treated with each polymer bound phosphonium bromide salt in water, **Scheme 162**.

It was very satisfying to find that both immobilised bromide sources racemised the α-bromo methyl ester **171** in an aqueous system. TG-PPh_3Br unfortunately racemised the α-bromo acid **172** and would not be appropriate for a *dynamic resolution*. The Wang based phosphonium bromide racemised the starting material, methyl-α-bromo phenyl acetate **171** in the presence of α-bromo phenyl acetic acid **172** at room temperature with no side reaction taking place, an

ideal racemisation to be combined with the CLEC catalysed *kinetic resolution* to produce a *dynamic resolution*.



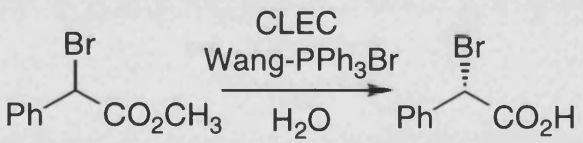
(R)-171 **(S)-172** **+/- 171** **(S)-172**

Bromide Source	Time	(ester)[b]		(acid)[b]	
	hrs.	init. ee%	final ee%	init ee%	final ee%
TG-PPh_3Br [a]	2	44	3	61	44
Wang-PPh_3Br [a]	2	43	1	61	58

Reactions carried out using < 0.5 equivalents of bromide. a) Resin supported phosphonium salts must be used as soon as possible after preparation. Though they can be stored below 0 °C activity does decrease after ~10 hours. b) Enantiomeric excess determined by HPLC analysis.

Scheme 162.

Our thiol battered noses began to smell the perfume of success and Wang-PPh_3Br was added in sub-stoichiometric amount to the CLEC catalysed hydrolysis, **Scheme 163**.



171 **(S)-172**

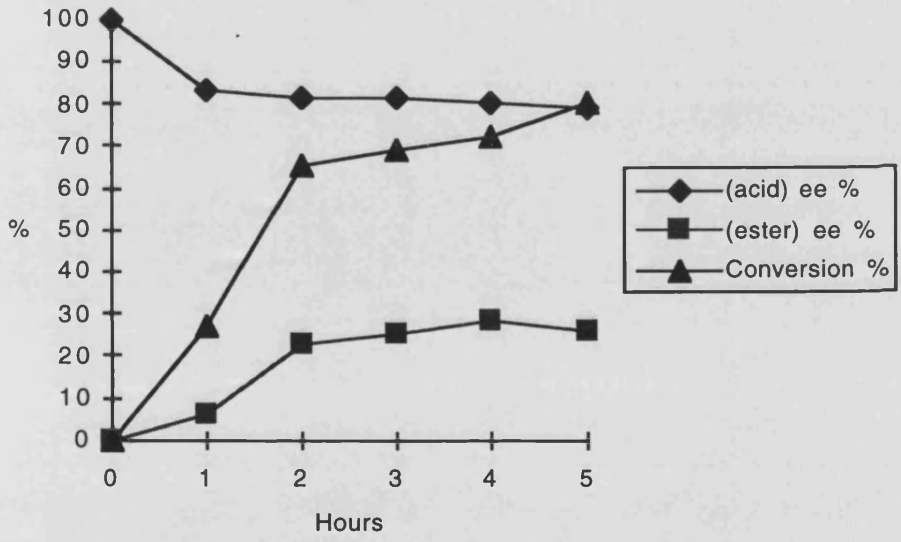
Conditions	Time	Conv.	(S)-acid[a]	(R)-ester[a]	(acid)
	hrs.	%	ee %	ee %	yield %
0.5 eq Wang-PPh_3Br	4.5	80	79	26	78
0.4 eq Wang-PPh_3Br	4	86	70	28	84

a) Enantiomeric excess determined by HPLC analysis, stereochemical configuration determined by optical rotation.

Scheme 163.

Here we see the *dynamic resolution* of methyl-α-bromo phenyl acetate **171** achieved by CLEC-CR and Wang-PPh_3Br in water at room temperature carried out over 4 hours. The (S)-α-bromo acid **172** was isolated in up to 79% enantiomeric excess at conversions up to 86 % and yields up to 84%. The enantiomeric excess of the starting material, methyl-α-bromo phenyl acetate **171** never rose above 28% during the course of the reaction.

The course of the reaction was monitored by HPLC analysis and the results are shown below, **Scheme 164**.



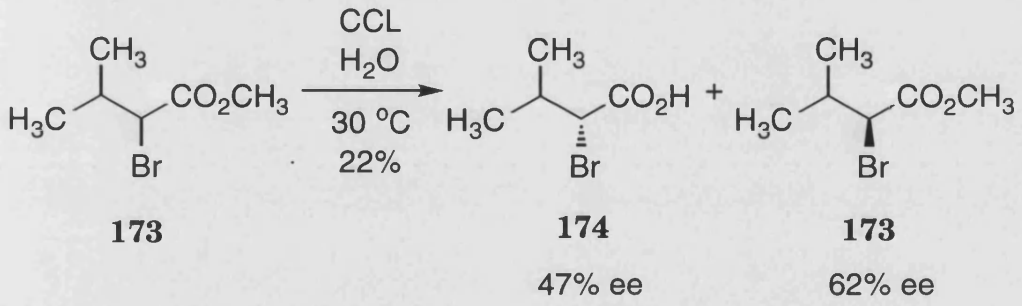
Scheme 164.

Here we can see that conversion rose to 65% after the first 2 hours and only rose a further 15% to 80% in the following 2.5 hours. The enantiomeric excess of (R)-methyl-α-bromo phenyl acetate **171** rose to 23% after 2 hours and was maintained around that value throughout the reaction. The enantiomeric excess of (S)-α-bromo phenyl acetic acid **172** produced remained at ~ 80 % throughout the course of the reaction and no racemisation of the acid by the immobilised bromide source was observed. A *dynamic resolution*.

5.8 Conclusions.

In this Chapter the enzymatic *kinetic resolution* of a selection of α-bromo esters was investigated. The substrates were resolved in moderate to good enantiomeric excesses.

Methyl-2-bromo-3-methyl butanoate **173** was hydrolysed by CCL at 30 °C over 18 hours to produce the α-bromo acid **174** in 47% enantiomeric excess at 22% conversion. The remaining methyl ester **173** was recovered in 62% enantiomeric excess, **Scheme 165**.

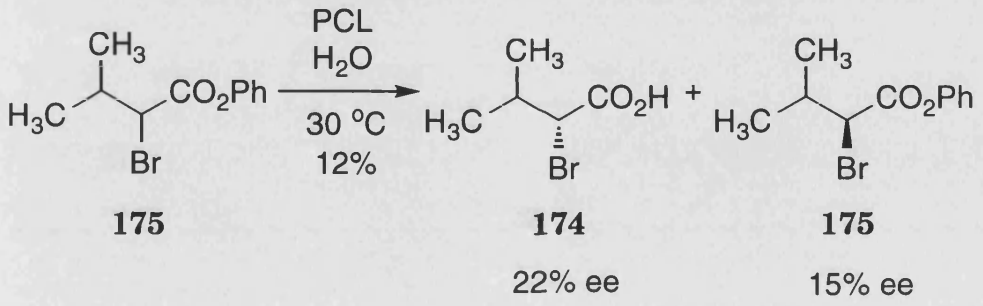


173 **174** **173**

47% ee 62% ee

Scheme 165.

The bulky phenyl ester **175**, **Scheme 166**, was hydrolysed at a much slower rate. Similar conversions were only obtained after a longer reaction time (18 hrs Vs 48 hrs), and in all cases the phenyl ester was hydrolysed with lower stereoselectivity.

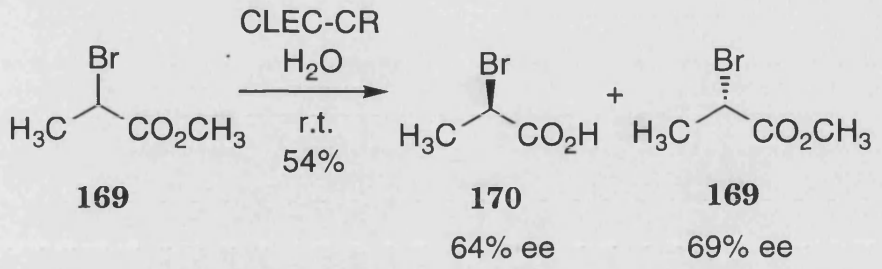


175 **174** **175**

22% ee 15% ee

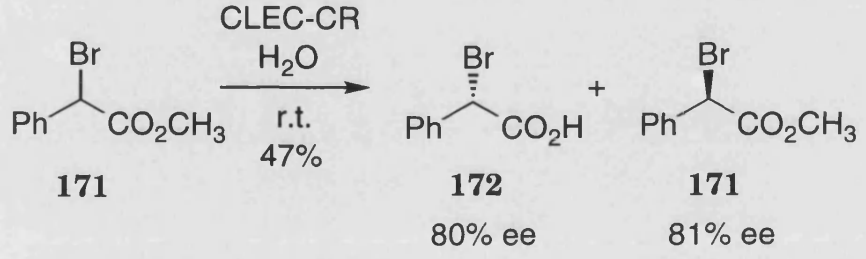
Scheme 166.

Methyl-α-bromo propionate **169** was resolved using CLEC-CR to produce α-bromo propionic acid **170** in 64% enantiomeric excess at 54% conversion over 1.5 hours at room temperature, **Scheme 167**.



Scheme 167.

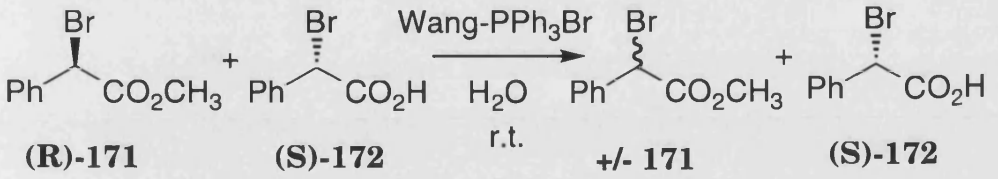
Methyl-α-bromo phenyl acetate **171** was also resolved by CLEC-CR to produce the (S)-α-bromo acid **172** in 80% enantiomeric excess at 47% conversion at room temperature. The remaining (R)-methyl-α-bromo phenyl acetate **171** was isolated in 81% enantiomeric excess, **Scheme 168**.



Scheme 168.

Selective racemisation of (R)-methyl-α-bromo phenyl acetate **171** was achieved using a series of quaternary ammonium or phosphonium bromide salts in aqueous or organic/aqueous reaction media. When combined with the enzymatic *kinetic resolution* a side reaction occurred resulting in reduced conversion.

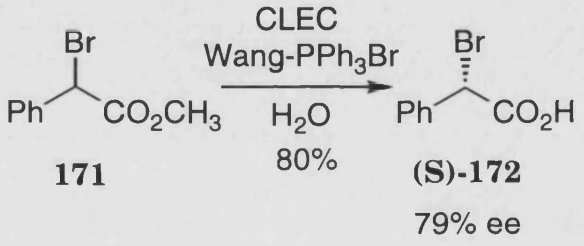
'Clean' selective racemisation was effected using a polymer bound quaternised phosphonium bromide salt, **Scheme 169**.



(R)-171 **(S)-172** **+/- 171** **(S)-172**

Scheme 169.

Wang-PPh_3Br selectively racemised the (R)-α-bromo methyl ester **171** in the presence of (S)-α-bromo phenyl acetic acid **172** in an aqueous system at room temperature and neutral pH. This racemising agent was combined with the CLEC-CR catalysed *kinetic resolution* to achieve the *dynamic resolution* of methyl-α-bromo phenyl acetate **171**, **Scheme 170**.



171 **(S)-172**
79% ee

Scheme 170.

The produced (S)-α-bromo phenyl acetic acid **172** was isolated from the reaction in up to 79% enantiomeric excess at 80% conversion and 78% overall yield providing an effective and synthetically useful *dynamic resolution*.

6.0 Experimental Section.

6.1 General Experimental.

Commercially available solvents and reagents were used throughout without further purification, except for those outlined below which were purified as described.

Petroleum ether refers to the fraction boiling between 40 °C and 60 °C and was distilled through a 36 cm Vigreux column before use. Diethyl ether was dried by storing over sodium wire for several days. Dichloromethane (DCM) was distilled from phosphorus pentoxide.

Analytical thin layer chromatography was carried out on aluminium or plastic backed plates coated with Merck Kieselgel 60 GF_{254}. Plates were visualised under light (254 nm) or by staining with phosphomolybdic acid or potassium permanganate reagent, followed by heating. Flash chromatography was carried out using Merck Kieselgel 60 H silica or Sorbil C 60 silica gel. Pressure was applied to the column head by either hand bellows or compressed air. Samples were applied pre-adsorbed on silica or as a saturated solution in an appropriate solvent. Preparatory thin layer chromatography was carried out using glass backed plates coated with Merck Kieselgel 60 GF_{254}. Samples were coated onto the plate as a DCM solution.

IR spectra were recorded in the range 4000-600 cm^{-1} using a Nicolet FT-205 spectrometer with internal calibration. Spectra were recorded as neat samples or as a mull (Nujol).

^{1}H and ^{13}C NMR spectra were recorded using Bruker AC-250, DPX400, Joel GX 270, EX 400. ^{1}H spectra were referenced against TMS at 0 ppm. Signals were described as singlets (s), doublets (d), quartets (q), doublet of doublets (dd) etc. High and low resolution mass spectra were recorded on a Kratos MS80 instrument.

Melting points were recorded using an electrothermal digital melting point apparatus and are uncorrected.

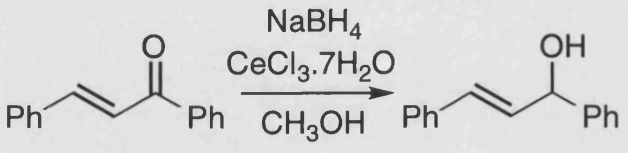
Optical rotations were recorded on an Optical Activity AA-10 automatic polarimeter.

Gas chromatography was carried out using Fisons GC8000 series equipment and Pye Unicam GCD chromatographs.

HPLC analysis was carried out using Thermoseparation Products Spectra series equipment.

6.2 Chapter 2 Experimental.

1, 3 - Diphenyl - 2 - propenol. [82]



42 **43**

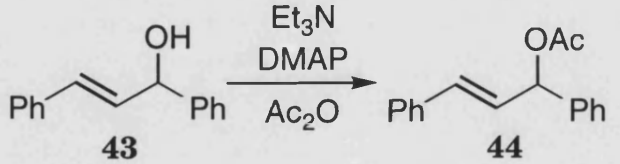
Chalcone (24 mmol, 5 g) was suspended in a solution of cerium chloride heptahydrate (1.1 eq, 26.6 mmol, 9.85 g) and methanol (50 ml) and cooled to 0 °C. Sodium borohydride (1.1 eq, 26.6 mmol, 1 g) was added with stirring to the reaction mixture.

Once all starting material had been consumed the solvent was removed *in vacuo* and extracted into ethyl acetate (3 x 50 ml), washed with water (50 ml), brine (30 ml), dried over $MgSO_4$, and filtered. The solvent was removed *in vacuo* and purified by flash chromatography (30% diethyl ether / petroleum ether).

Pale white solid (82%, M. pt 51.6 °C).

$\vartheta_{max.}$ / $^{cm\ -1}$ 3441; δ_H (250 MHz, $CDCl_3$) 5.35 (1H, d, J=6.4, C<u>H</u>OH), 6.37 (1H, dd, J=6.4, J=15.8, =C<u>H</u>), 6.67 (1H, d, J=15.8, =C<u>H</u>), 7.13 - 7.39 (10H, m, Ar<u>H</u>); δ_C (62. 5 MHz, $CDCl_3$) 75.1 (<u>C</u>H), 126.3 (=<u>C</u>H), 127 (C<u>H</u>), 127.7 - 141.4 (<u>Ar-C</u>); *m/z* (EI) 210 (34), 105 (100).

1, 3 - Diphenyl propenyl acetate. [83]



43 **44**

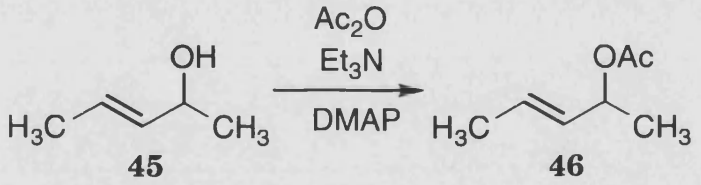
Allylic alcohol (22.5 mmol, 4.8 g) was dissolved in triethylamine (1.5 eq, 34.2 mmol, 3.45 g) and acetic anhydride (1.5 eq, 27 mmol, 2.78 g) with 2-3 crystals of DMAP. The reaction was stirred under nitrogen at room temperature until all starting material was consumed.

The reaction mixture was diluted with diethyl ether (20 ml) and washed with water (2 x 50 ml). The organic layer was collected and dried ($MgSO_4$), filtered, concentrated *in vacuo*, and purified by flash chromatography (20 % diethyl ether / petroleum ether) to yield **44**.

Yellow oil (76%).

$\vartheta_{max..}$ / $^{cm-1}$ 1737; δ_H (250 MHz, $CDCl_3$), 2.08 (3H, s, C(O)C<u>H</u>$_3$), 6.31 - 6.46 (2H, m, 2 x C<u>H</u>), 6.63 (1H, d, J=15.6, C<u>H</u>), 7.18 - 7.38 (10H, m, Ar<u>H</u>); δ_C (62.5 MHz, $CDCl_3$), 21.3 (<u>C</u>H$_3$), 76 (<u>C</u>H), 126.7 (<u>C</u>H), 127 (<u>C</u>H), 127.9 - 129.1 (<u>Ar-C</u>), 169.9 (<u>C</u>=O); *m / z* (EI) 252 (16), 191 (100), 209 (43).

4-Acetoxy penten-2-ene. [84]



45 **46**

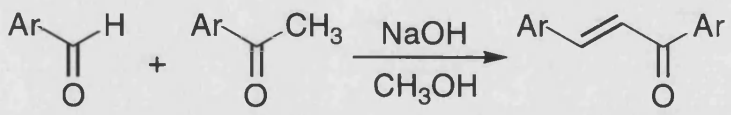
Penten-2-ol (11.6 mmol, 1 g) in triethylamine (1.5 eq, 17.4 mmol, 1.76 g) and acetic anhydride (1.2 eq, 13.9 mmol, 1.4 g) along with 3-4 crystals of DMAP were stirred at room temperature under nitrogen until all starting material was consumed.

The reaction mixture was diluted with diethyl ether (10 ml) and washed with water (15 ml). The organic layer was collected and dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield the allylic acetate. The product was purified by flash chromatography (20% diethyl ether / petroleum ether).

Light colourless oil (92%).

$\vartheta_{max.}$ / $^{cm-1}$ 1241, 1736; δ_H (250 MHz, $CDCl_3$) 1.24 (3H, d, J=5.5, CHC<u>H</u>$_3$), 1.67 (3H, d, J= 5.4, C<u>H</u>$_3$CH), 1.99 (3H, s, C(O)C<u>H</u>$_3$), 5.25 (1H, dq, J=7.5, J= 6.2, CH$_3$C<u>H</u>=CH), 5.27 (1H, dq, J=5.4, J=6.2, CH$_3$C<u>H</u>), 5.67 (1H, dd, J=6.2, J=5.4, CH=C<u>H</u>CH); δ_C (62.5 MHz, $CDCl_3$) 17.4 (<u>C</u>H$_3$), 20.1 (<u>C</u>H$_3$), 21.2 (<u>C</u>H$_3$), 70.9 (<u>C</u>H), 127.8 (=<u>C</u>H), 130.7 (<u>C</u>H=), 171.2 (<u>C</u>=O); *m / z* (EI) 127 (12), 28 (100).

α - β **Unsaturated ketones.**



47 **48** **49**

Ar = m-$(CH_3)C_6H_4$
Ar = m-$(OCH_3)C_6H_4$

Aldehyde (22 mmol) and ketone (1 eq, 22 mmol) were dissolved in methanol (20 ml) with 5-6 pellets of NaOH and stirred at room temp for 12 hrs.

The methanolic solution was diluted with diethyl ether (60 ml) and washed with water (2 x 50 ml), brine (30 ml), dried ($MgSO_4$), and filtered. The solvent was removed *in vacuo* to yield the condensation product.

m-Methoxy-1,3-diphenyl prop-2-en-1-one. [85]

Opaque green viscous oil (62 %).

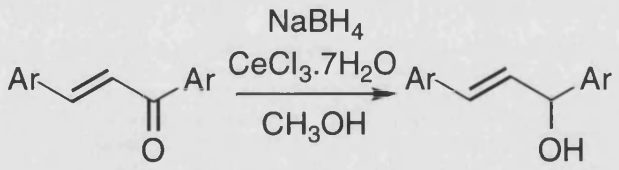
$\vartheta_{max.}$ / $^{cm-1}$ 1665; δ_H (250 MHz, $CDCl_3$) 3.83 (3H, s, $OC\underline{H}_3$), 3.86 (3H, s, $OC\underline{H}_3$), 7.39 (1H, d, J=15.7, CH=C<u>H</u>), 7.76 (1H, d, J=15.7, C(O)C<u>H</u>), 7.42 - 7.57 (8H, m, C<u>H</u>); δ_C (62.5 MHz, $CDCl_3$) 55.1 ($O\underline{C}H_3$), 55.3 ($O\underline{C}H_3$), 112.8 (=<u>C</u>H), 113.2 (=<u>C</u>H), 116.2 - 119.8 (<u>Ar-C</u>), 190 (C=O); *m/z* (EI) 268 (37), 206 (57).

m-Methyl-1,3-diphenyl prop-2-en-1-one. [86]

Opaque orange oil (58 %).

$\vartheta_{max.}$ / $^{cm-1}$ 1607; δ_H (250 MHz, $CDCl_3$) 2.36 (3H, s, $C\underline{H}_3$), 2.41 (3H, s, $C\underline{H}_3$), 7.41 (1H, d, J=15.4, CH=C<u>H</u>), 7.71 (1H, d, J=15.4, C(O)C<u>H</u>), 7.22 - 7.4 (10H, m, Ar<u>H</u>); δ_C (62.5 MHz, $CDCl_3$) 21.3 ($\underline{C}H_3$), 21.4 ($\underline{C}H_3$), 125.3 (=<u>C</u>H), 125.6 (=<u>C</u>H), 127.6 - 138.5 (<u>Ar-C</u>), 192 (<u>C</u>=O); *m/z* (EI) 236 (32), 206 (52).

Allylic alcohols.



49 **50**

Ar = m-$(CH_3)C_6H_4$
Ar = m-$(OCH_3)C_6H_4$

Cerium chloride heptahydrate (1.1 eq, 10.2 mmol, 3.8 g) was added to the α, β-unsaturated ketone (9.27 mmol, 2.5 g) in methanol (20 ml). To this sodium borohydride (1.1 eq, 10.2 mmol, 0.4 g) was added with stirring at room temperature until all starting material was consumed. The reaction mixture was diluted with ether (100 ml) and washed with water (50 ml). The organic layer was collected and dried ($MgSO_4$), filtered, and the solvent removed *in vacuo*.

m-Methoxy-1,3-diphenyl prop-2-en-1-ol. [99]

Yellow oil (98%)

$\vartheta_{max.}$ / cm-1 3423; δ_H (250 MHz, $CDCl_3$) 3.74 (3H, s, $OC\underline{H}_3$), 3.76 (3H, s, $OC\underline{H}_3$), 5.27 (1H, d, J=6.7, $C\underline{H}OH$), 6.32 (1H, dd, J=15.6, J=6.7, $C\underline{H}$=CH), 6.57 (1H, d, J=15.6, CH=$C\underline{H}$), 6.89 -7.23 (8H, m, Ar$\underline{H}$); δ_C (62.5 MHz, $CDCl_3$) 55.2 ($O\underline{C}H_3$), 55.3 ($O\underline{C}H_3$), 74.7 ($\underline{C}$HOH), 130.5 (=$\underline{C}$H), 131.7 (=$\underline{C}$H), 111.6 - 119.0 (Ar-$\underline{C}$); *m/z* (EI) M^+ 270 (28), 136 (100).

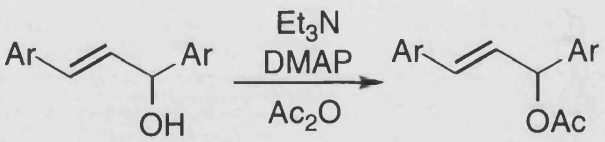
m-Methyl-1,3-diphenyl prop-2-en-1-ol. [99]

Yellow oil (93%)

V max. / cm-1 1605; δ_H (400 MHz, $CDCl_3$) 2.29 (3H, s, $C\underline{H}_3$), 2.32 (3H, s, $C\underline{H}_3$), 5.28 (1H, d, J=7.5, OHC$\underline{H}$), 6.32 (1H, dd, J=7.5, J=15.9, C$\underline{H}$=CH), 6.61 (1H, d, J=15.9, CH=C$\underline{H}$), 7.01 - 7.23 (8H, m, Ar$\underline{H}$); δ_C

(100 MHz, $CDCl_3$) 21.1 (<u>C</u>H_3), 21.2 (<u>C</u>H_3), 75.3 (HO<u>C</u>), 123.5 (=<u>C</u>H), 123.9 (=<u>C</u>H), 124.2 - 138.3 (<u>Ar-C</u>); *m/z* (EI) 238 (21), 208 (61).

Allylic Acetates.



50 **51**

Ar = m-$(CH_3)C_6H_4$
Ar = m-$(OCH_3)C_6H_4$

Allylic alcohol (12.8 mmol, 3 g) in triethylamine (1.5 eq, 19.2 mmol, 1.9 g), acetic anhydride (1 eq, 12.8 mmol, 1.3 g) and 2 - 3 crystals of DMAP were stirred under nitrogen at 0 °C. After 3 hours the reaction was allowed to warm to room temperature and stirred for an additional 1 hour until all starting material was consumed.

The reaction mixture was diluted with diethyl ether (100 ml) and washed with water (70 ml), and brine (70 ml). The organic layer was separated, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The product acetate was purified using flash chromatography (20 % diethyl ether / petroleum ether).

m-Methoxy-1,3-diphenyl-1-acetoxyprop-2-ene. [98]

Yellow oil (80%).

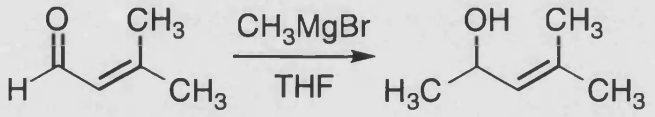
$\vartheta_{max.}$ / cm^{-1} 1737; δ_H (250 MHz, $CDCl_3$) 2.15 (3H, s, $COCH_3$), 3.76 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 6.35 (1H, dd, J=15.5, J=6.9, CH=C<u>H</u>), 6.42 (1H, d, J=7.0, (OAc)C<u>H</u>), 6.62 (1H, d, J=15.5, C<u>H</u>=CH), 6.87 - 7.23 (8H, m, Ar<u>H</u>); δ_C (62.5 MHz, $CDCl_3$) 21.4 ($COCH_3$), 55.3 (2 x OCH_3), 75.6 (<u>C</u>H(OAc)), 111.2 - 119.1 (<u>Ar-C</u>), 128.1 (=<u>C</u>H), 132.7 (=<u>C</u>H), 171.0 (<u>C</u>=O); *m/z* (EI) 253 (68), 191 (100).

m-Methyl-1,3-diphenyl-1-acetoxyprop-2-ene. [98]

Pale yellow oil (78 %).

$\vartheta_{max.}$ / $^{cm-1}$ 1761; δ_H (250 MHz, $CDCl_3$) 2.21 (3H, s, C(O)C<u>H</u>$_3$), 2.41 (3H, s, C<u>H</u>$_3$), 2.42 (3H, s, C<u>H</u>$_3$), 6.39 (1H, dd, J=7.1, J=15.4, CH=C<u>H</u>), 6.48 (1H, d, J=7.1, C<u>H</u>=CH), 6.69 (1H, d, J=15.4, (OAc)C<u>H</u>), 7.14 - 7.35 (8H, m, Ar<u>H</u>); δ_C (100 MHz, $CDCl_3$) 20.3 (<u>C</u>H$_3$), 20.5 (<u>C</u>H$_3$), 76.0 ((OAc)<u>C</u>H$_3$), 123.2 - 127.6 (<u>Ar-C</u>), 128.4 (=CH), 130.7 (=CH), 169 (<u>C</u>=O); *m/z* (EI) 280 (22), 250 (29).

4-Methyl-3-penten-2-ol. [101]



52 **53**

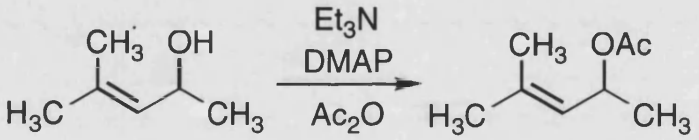
3-Methyl-2-butenal (5 mmol, 0.45 g), in dry THF (10 ml) under nitrogen at 0 °C was treated with CH3MgBr (1.5 eq, 7.5 mmol, 2.5 ml) and stirred at 0 °C for 30 minutes. The reaction was allowed to warm to room temperature and stirred for an additional 1.5 hrs.

The reaction mixture was diluted with EtOAc (10 ml), quenched with saturated NH_4Cl (10 ml), washed with water (10 ml), dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield the alcohol **53**.

Colourless oil (89%).

ϑ_{max} / $^{cm-1}$ 3429; δ_H (250 MHz, $CDCl_3$) 1.32 (3H, d, J=5.2, C<u>H</u>$_3$CH), 1.62 (3H, s, C(CH$_3$)C<u>H</u>$_3$), 1.64 (3H, s, C(CH$_3$)C<u>H</u>$_3$), 5.21 (1H, dq, J=5.2, J=7.1, CH$_3$C<u>H</u>), 5.49 (1H, d, J=7.1, CHC<u>H</u>=).

4-Methyl-2-acetoxypent-3-ene. [100]



53 **54**

Allylic alcohol (0.7 g, 7 mmol) was added to triethylamine (1.5 eq, 10.5 mmol, 1 g), Ac_2O (1.1 eq, 7.7 mmol, 0.78 g) and 1-2 crystals of DMAP.

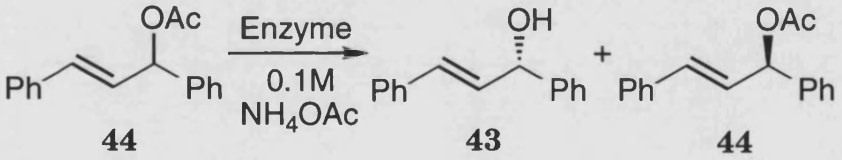
The reaction mixture was stirred under N_2 at room temperature for 2 hours.

The reaction mixture was washed with water (10 ml), extracted into diethyl ether (15 ml), separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The allylic acetate **54** was purified using flash chromatography (20% diethyl ether / petroleum ether).

yellow oil (87%).

$\vartheta_{max.}$ / $^{cm-1}$ 1797; δ_H (250 MHz, $CDCl_3$) 1.47 (3H, d, J=5.2, $C\underline{H}_3$), 1.64 (3H, s, $C\underline{H}_3$), 1.69 (3H, s, $C\underline{H}_3$), 2.02 (3H, s, $C(O)C\underline{H}_3$), 5.29 (1H, m, $CH=C\underline{H}$), 5.69 (1H, m, $=CHC\underline{H}CH_3$); δ_C (62.5 MHz, $CDCl_3$) 21.3 ($\underline{C}H_3$), 21.4 ($\underline{C}H_3$), 24.5 ($\underline{C}H_3$), 71.2 ($C(O)\underline{C}H_3$), 76 ($\underline{C}H$), 126.7 ($\underline{C}H$), 127 ($\underline{C}H$), 172.1 ($\underline{C}=O$); *M/Z* EI) 252 (15), 191 (100), 209 (43).

Enzymatic hydrolysis of 1,3-diphenyl-propenyl acetate.

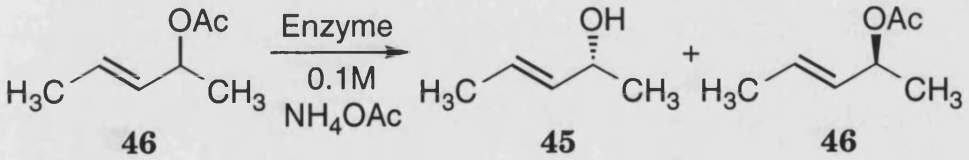


44 **43** **44**

Allylic acetate (1 mmol) in ammonium acetate buffer (0.1M, 5 ml, pH 7.0) was treated with enzyme (25 mg) and stirred at 38 °C. The pH of the reaction was monitored and NaOH (0.1M) was periodically added to maintain a neutral pH.

The reaction products were extracted into diethyl ether and filtered through a silica plug. Removal of the solvent *in vacuo* yielded the allylic acetate and alcohol.

Enzymatic hydrolysis of 3-acetoxy penten-2-ene.

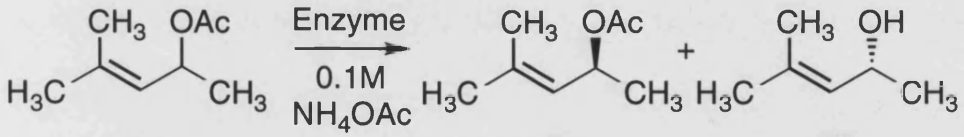


46 **45** **46**

Allylic acetate (2 mmol, 0.26 g) in ammonium acetate buffer (0.1M, 6 ml, pH 7.0) was treated with the enzyme (50 mg) and the reaction was maintained at 40 °C for 2 days.

The reaction mixture was shaken with EtOAc (5 ml) and filtered through a plug of silica. The solvent was removed *in vacuo* to yield the allylic acetate and alcohol.

Enzymatic hydrolysis of 4-methyl-2-acetoxypent-3-ene.

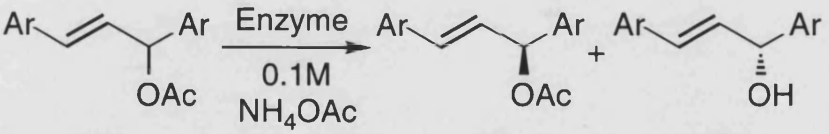


54 **54** **53**

Allylic acetate (0.71 g, 5 mmol) in ammonium acetate buffer (0.1M, 2 ml, pH 7.0) was treated with the enzyme (25 mg). The temperature of the reaction was held at 40 °C for 6 days.

The reaction mixture was shaken with diethyl ether (10 ml), filtered through a plug of silica, and concentrated *in vacuo* to yield the allylic acetate and alcohol.

Enzymatic hydrolysis of m-methoxy-1,3-diphenyl-1-acetoxyprop-2-ene and m-methyl-1,3-diphenyl-1-acetoxyprop-2-ene.



51 **51** **50**

Ar = m-$(CH_3)C_6H_4$

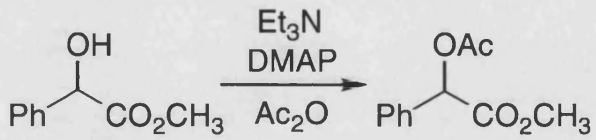
Ar = m-$(OCH_3)C_6H_4$

The allylic acetate (1 mmol, 0.25 g) in ammonium acetate buffer (0.1M, 6 ml, pH 7.0) was treated with the enzyme (20 mg) at 40 °C for 2 days.

The reaction products were extracted into EtOAc (4 ml) and filtered through a plug of silica. Removal of the solvent *in vacuo* yielded the allylic acetate and alcohol.

6.3 Chapter 3 Experimental.

Acetoxy methyl mandelate. [87]



103 **104**

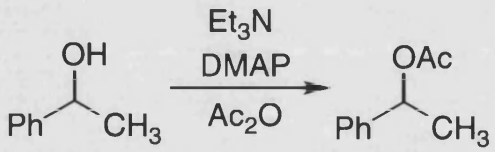
Methyl mandelate (0.5 g, 3 mmol) in triethylamine (1.5 eq, 4.5 mmol, 0.45 g), Ac_2O (1.1 eq, 3.3 mmol, 0.35 g) and 1-2 crystals of DMAP was stirred at room temperature under N_2 until all starting material was consumed.

The reaction mixture was diluted with diethyl ether (20 ml), washed with water (20 ml), separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The acetate was purified by flash chromatography (20% ether / petroleum ether).

clear oil (87%).

$\vartheta_{max.}$ / cm^{-1} 1687; δ_H (250 MHz, $CDCl_3$) 2.17 (3H, s, $CO_2C\underline{H}_3$), 3.65 (3H, s, $CO_2C\underline{H}_3$), 5.90 (1H, s, C<u>H</u>), 7.31 - 7.46 (5H, m, Ar<u>H</u>);

1-Phenethyl acetate. [88]



101 **102**

1-Phenethyl alcohol (0.5 g, 4.1 mmol) in triethylamine (1.5 eq, 6.15 mmol, 0.62 g), Ac_2O (1.1 eq, 4.5 mmol, 0.46 g), and 2-3 crystals of DMAP was stirred under nitrogen at room temperature for 4 hours.

Once all starting material was consumed the reaction was diluted with diethyl ether (20 ml), washed with water (20 ml), and the organic layer collected. This was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The acetate was purified by flash chromatography (20% diethyl ether / petroleum ether) to yield 1-phenethyl acetate.

Colourless oil (96%).

δ_H (250 MHz, $CDCl_3$) 1.55 (3H, d, J=6.6, C<u>H</u>$_3$), 2.0 (3H, s, C<u>H</u>$_3$), 5.87 (1H, q, J=6.6, C<u>H</u>), 7.25-7.37 (5H, m, Ar<u>H</u>); δ_C (68 MHz, $CDCl_3$) 21.1 (<u>C</u>H$_3$), 22.0 (C(O)<u>C</u>H$_3$), 73.3 (H<u>C</u>(OAc)), 126.0 (<u>C</u>H), 127.2 (<u>C</u>H), 128.0 (<u>C</u>H), 128.5 (<u>C</u>H), 129 (<u>C</u>H), 137 (<u>C</u>), 171 (<u>C</u>=O).

1-Phenyl-1-acetoxy propane. [89]

99 **100**

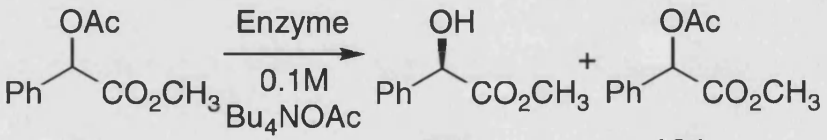
1-Phenyl-1-propanol (1 g, 7.36 mmol) in triethylamine (1.1 eq, 8.08 mmol, 0.82 g) Ac_2O (1.1 eq, 8.08 mmol, 0.8 g) and 1-2 crystals DMAP. The reaction mixture was stirred under N_2 at room temperature for 4 hours.

The reaction mixture was diluted with diethyl ether (20 ml), washed with water (30 ml), separated, dried ($MgSO_4$), filtered and concentrated *in vacuo*. The acetate product was purified by flash chromatography (20% ether / petroleum ether).

Colourless oil (85%).

$\vartheta_{max.}$ / $^{cm-1}$ 1780; δ_H (250 MHz, $CDCl_3$) 0.88 (2H, t, J=7.5, C<u>H</u>$_3$), 1.86 (2H, m, J=7.7, C<u>H</u>$_2$), 2.21 (3H, s, C(O)C<u>H</u>$_3$), 5.66 (1H, t, J=7.5, C<u>H</u>), 7.31 - 7.36 (5H, m, Ar<u>H</u>); δ_C (68 MHz, $CDCl_3$) 9.9 (<u>C</u>H$_3$), 21.1 (<u>C</u>H$_3$), 29.0 (<u>C</u>H$_2$), 76.4 (<u>C</u>H), 126.2 - 127.3 (<u>Ar-C</u>), 170.7 (<u>C</u>=O)

Enzymatic hydrolysis of 2-acetoxy methyl mandelate.

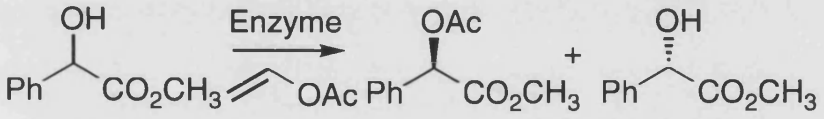


104 **103** **104**

Acetate (100 µl, 0.5 mmol) in tetrabutylammonium acetate buffer (0.1M, 1 ml, pH 7.0) was treated with the enzyme (5 mg) and stirred at 55 °C over 48 hrs.

The reaction mixture was shaken with diethyl ether and filtered through a plug of silica. Removal of the solvent *in vacuo* yielded the alcohol and acetate.

Enzymatic acetylation of methyl mandelate.

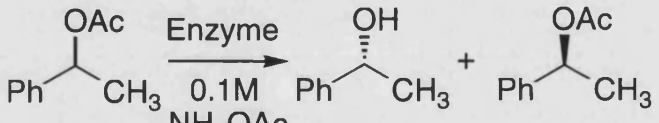


103 **104** **103**

Methyl mandelate (1 g, 6 mmol) in vinyl acetate (6 ml) was stirred at 40 °C over 3 days with the enzyme (25 mg)

The reaction was extracted with diethyl ether (10 ml) and filtered through a plug of silica. The solvent was removed *in vacuo* and the products separated by flash chromatography (30% ether / petroleum ether).

Enzymatic hydrolysis of 1-phenethyl acetate.

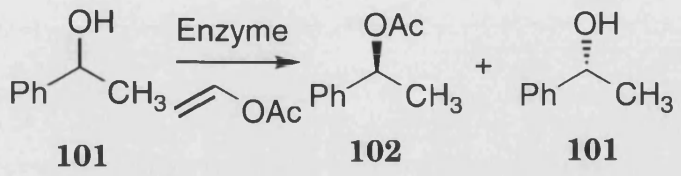


102 **101** **102**

Phenethyl acetate (0.6 g, 1 mmol) in ammonium acetate buffer (0.1M, 5 ml, pH 7.0) was treated with the enzyme (20 mg) and stirred at room temperature over 3 days.

The reaction mixture was diluted with ethyl acetate and filtered through a plug of silica. Removal of the solvent *in vacuo* yielded the alcohol and acetate. Products were separated using flash chromatography (20% diethyl ether / petroleum ether).

Enzymatic acetylation of 1-phenethyl alcohol.

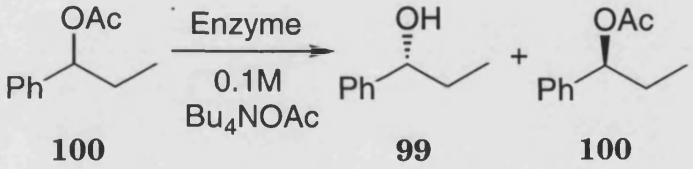


101 **102** **101**

Phenethyl alcohol (3 g, 24.5 mmol) in vinyl acetate (40 ml) and enzyme (40 mg) was stirred under N_2 at room temperature for 4 days.

The vinyl acetate was removed *in vacuo* and the reaction mixture diluted with diethyl ether (40 ml), washed with water (40 ml), separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The reaction products were separated by flash chromatography (20% ether / petroleum ether).

Enzymatic hydrolysis of 1-Phenyl-1-acetoxy propane.

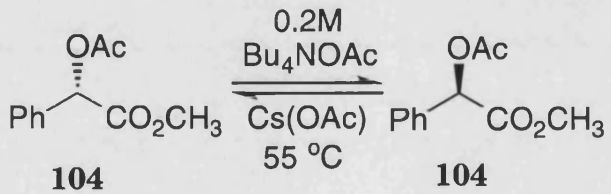


100 **99** **100**

1-Phenyl-1-acetoxypropane (100 µl, 0.6 mmol) was emulsified in tetrabutylammonium acetate buffer (0.2M, 1 ml, pH 7.0). Enzyme (10 mg) was added and the reaction stirred at room temperature for 2 days.

The reaction mixture was filtered through silica and concentrated *in vacuo* to afford the acetate and alcohol.

Racemisation of acetoxy methyl mandelate.



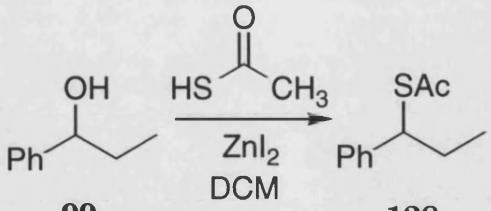
104 **104**

(S)-Acetoxy methyl mandelate (100 µl, 0.5 mmol) was emulsified in tetrabutyl ammonium acetate (0.2M, 0.8 ml). Caesium acetate (20 mol% %) was added and the mixture stirred at 55 °C for 2 days.

The reaction mixture was extracted with diethyl ether, filtered through a plug of silica, and concentrated *in vacuo* to afford racemic acetoxy methyl mandelate.

6.4 Chapter 4 Experimental.

1-Phenyl-1-thioacetoxy propane. [91]



99 **128**

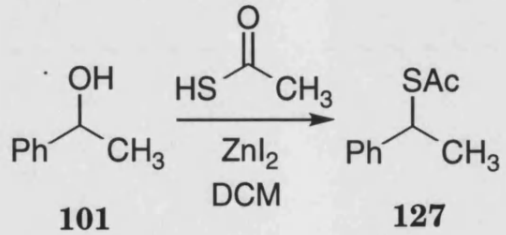
1-Phenyl propan-1-ol (3 g, 22 mmol) in dichloromethane (100 ml) was stirred with ZnI_2 (0.5 eq, 11 mmol, 3.5 g). To this thiolacetic acid (1.1 eq, 24 mmol, 1.9 ml) was added and the reaction mixture stirred for 4 hours at room temperature.

The reaction mixture was washed with water (5 x 30 ml) and diluted with dichloromethane (40 ml). The organic layer was collected, filtered through a plug of celite, and concentrated *in vacuo*. The product was purified by flash chromatography (20 % diethyl ether / petroleum ether).

Colourless oil (69%).

$\vartheta_{max.}$ / cm^{-1} 1702; δ_H (400 MHz, $CDCl_3$) 0.85 (3H, t, J=7.4, $CH_2C\underline{H}_3$), 1.35 - 156.2 (2H, m, $C\underline{H}_2CH_3$), 2.27 (3H, s, $C(O)C\underline{H}_3$), 4.44 (1H, t, J=7.4, C<u>H</u>), 7.19-7.34 (5H, m, Ar<u>H</u>); δ_C (100 MHz, $CDCl_3$) 19.1 ($\underline{C}H_3$), 39.4 ($\underline{C}H_2$), 44.6 (<u>C</u>H), 58.7 ($C(O)\underline{C}H_3$), 127.1 (2x<u>C</u>H), 127.3 (<u>C</u>H), 127.4 (2x<u>C</u>H), 128.1 (<u>C</u>), 195.4 (<u>C</u>=O).

Phenethyl thioacetate. [91]



101 **127**

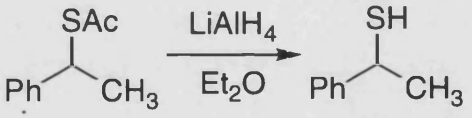
Phenethyl alcohol (1 g, 8 mmol) in dichloromethane (16 ml) was stirred with ZnI_2 (0.5 eq, 13.3 g). To this thiolacetic acid (1.2 eq, 9.8 mmol, 0.8 ml) was added and the reaction mixture stirred at room temperature for 4 hrs.

The reaction mixture was washed with water (4 x 20 ml) and diluted with dichloromethane (20 ml). The organic layer was filtered through a plug of celite, collected, dried ($MgSO_4$), filtered and the solvent removed *in vacuo*. The product was purified by flash chromatography (30% diethyl ether / petroleum ether).

Colourless oil (86%)

$\vartheta_{max.}$ / cm^{-1} 1697; δ_H (250 MHz, $CDCl_3$) 1.65 (3H, s, J=7.4, $CHC\underline{H}_3$), 2.31 (3H, s, $COC\underline{H}_3$), 4.75 (1H, q, J=7.3, C<u>H</u>), 7.34 (5H, m, Ar<u>H</u>).

Phenethyl thiol. [92]



127 **120**

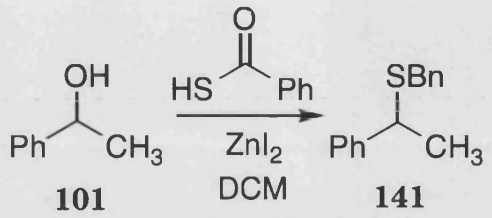
Phenethyl thioacetate (1.7 g, 9.3 mmol) in diethyl ether (20 ml) was treated with $LiAlH_4$ (1M soln. in Et_2O, 1.1 eq, 10.2 mmol, 10.2 ml) at 0 °C with stirring under a nitrogen atmosphere for 1 hr. The reaction mixture was allowed to warm to room temperature and stirred for an additional 1 hour.

The reaction was re-cooled to 0 °C and HCl (0.1M) was added until the white precipitate disappears. The reaction mixture was diluted with diethyl ether (20 ml), washed with water (30 ml), and the organic layer collected. This was dried ($MgSO_4$), filtered, and concentrated *in vacuo*, and the thiol purified using flash chromatography (10 diethyl ether / petroleum ether).

Colourless oil (96%).

$\vartheta_{max.}$ / cm-1 2968, 1489; δ_H (400 MHz, $CDCl_3$) 1.67 (3H, d, J=7.2, $C\underline{H}_3$), 1.97 (1H, d, J=5.2, S$\underline{H}$), 4.25 (1H, dq, J=5.2, 7.1, C$\underline{H}$), 7.31 (5H, m, Ar$\underline{H}$).

Phenethyl thiobenzoate. [91]



101 **141**

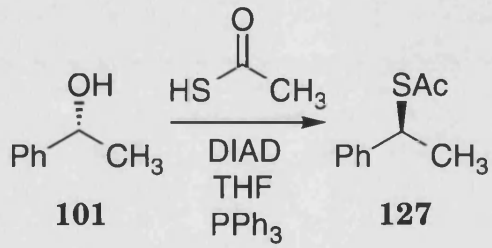
Phenethyl alcohol (1 g, 8 mmol) in dichloromethane (20 ml) was stirred with ZnI_2 (0.5 eq, 4 mmol, 1.3 g). Thiolbenzoic acid (1.2 eq, 9.8 mmol, 1.36 g, 1.6 ml) was added and the reaction mixture stirred at room temperature for 8 hours.

The reaction mixture was washed with water (30 ml) and filtered through a plug of celite. The solvent was removed *in vacuo* and the thiobenzoate was purified by flash chromatography (20 % diethyl ether / petroleum ether).

Pale yellow oil (61%).

$\vartheta_{max.}$ / cm-1 1687; δ_H (250 MHz, $CDCl_3$) 1.68 (3H, d, J=7.5, CHC$\underline{H}_3$), 4.88 (1H, q, J=7.5, C$\underline{H}$), 7.42 (8H, m, Ar$\underline{H}$), 7.95 (2H, m, C(O)Ar$\underline{H}$); δ_C (68 MHz, $CDCl_3$) 21.4 ($\underline{C}H_3$), 40.9 ($\underline{C}$H), 116.9 ($\underline{C}$H), 120.6 ($\underline{C}$H), 127.2 - 133.0 (Ar-$\underline{C}$), 191.4 ($\underline{C}$=O).

(S)-Phenethyl thioacetate. [93]



101 **127**

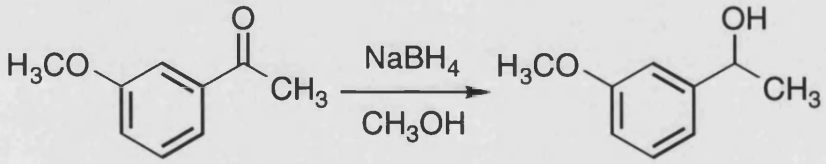
Diisopropylazodicarboxylate (1.29 ml, 6.6 mmol) was added dropwise to triphenyl phosphine (1.72 g, 6.54 mmol) in dry THF (12 ml) at 0 °C and allowed to stir under nitrogen for 30 minutes. (R)-Phenethyl alcohol (0.5 eq, 0.4 g, 3.28 mmol) and thiolacetic acid (470 µl, 6.5 mmol) were added dropwise to this solution and stirred at 0 °C for 1 hour then allowed to warm to room temperature. The mixture was stirred for an additional hour.

The reaction was washed with saturated aqueous $NaHCO_3$ (3 x 10 ml) and extracted with hexane. The organic layer was separated and filtered through a plug of celite, concentrated *in vacuo* to 0.5 % of initial volume and filtered through a plug of silica. The solvent was removed *in vacuo* and the product purified by flash chromatography (5% diethyl ether / hexane).

Colourless oil (81%).

$\vartheta_{max.}$ / cm^{-1} 2927, 1701; δ_H (250 MHz, $CDCl_3$) 1.59 (3H, d, J=7.1, $CHC\underline{H}_3$), 2.29 (3H, s, $SC(O)C\underline{H}_3$), 4.81 (1H, q, J=7.1, $C\underline{H}CH_3$), 7.40 - 7.48 (5H, m, Ar$\underline{H}$); $[\alpha]_D^{23}$ -284.2 ° (c = 0.8, DCM), lit: $[\alpha]_D^{23}$ -287.0 ° (c = 0.84, $CHCl_3$). [93]

m-Methoxy phenethyl alcohol. [94]



133 **136**

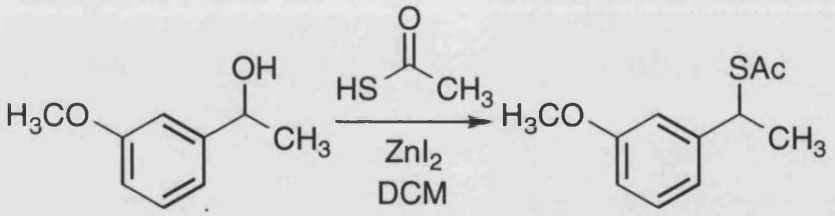
m-Methoxy acetophenone (2 g, 13.3 mmol) was dissolved in CH_3OH (20 ml). To this solution $NaBH_4$ (1.1 eq, 14.6 mmol, 0.56 g) was added slowly with stirring at 0 °C until all starting material was consumed.

The reaction mixture was extracted with diethyl ether (20 ml), washed with water (30 ml), and the organic layer separated. This was dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield alcohol. No further purification was required.

Colourless oil (96%)

$\vartheta_{max.}$ / cm^{-1} 2647; δ_H (250 MHz, $CDCl_3$) 1.46 (3H, d, J=5.3, C<u>H</u>$_3$), 3.78 (3H, s, OC<u>H</u>$_3$), 4.83 (1H, q, J=5.2, C<u>H</u>), 7.26 (5H, m, Ar<u>H</u>)

m-Methoxy phenethyl thioacetate.



136 **130**

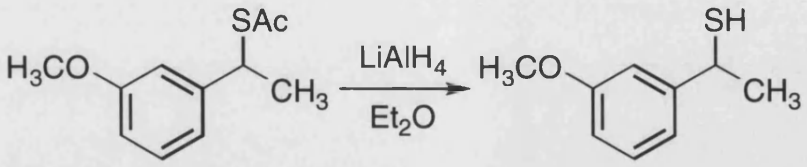
m-Methoxy phenethyl alcohol (3.6 g, 23.7 mmol) in dichloromethane (45 ml) was stirred with ZnI_2 (.5 eq, 11.8 mmol, 3.8 g) and treated with thiolacetic acid (1.2 eq, 28.4 mmol, 2.16 g, 2.3 ml) with stirring at room temperature for 4 hours.

The reaction mixture was washed with water (80 ml), and the organic layer collected. This was filtered through a plug of celite and concentrated *in vacuo* to yield the crude product. Purification was carried out by flash chromatography (10% diethyl ether / petroleum ether).

Colourless oil (87%).

$\vartheta_{max.}$ / cm^{-1} 1687; δ_H (250 MHz, $CDCl_3$) 1.64 (3H, d, J=7.5, C<u>H</u>$_3$), 2.28 (3H, s, C(O)C<u>H</u>$_3$), 3.79 (3H, s, OC<u>H</u>$_3$), 4.71 (1H, q, J=7.4, C<u>H</u>), 7.13 (5H, m, Ar<u>H</u>); δ_C (100 MHz, $CDCl_3$) 21.4 (<u>C</u>H$_3$), 30.1 (<u>C</u>H), 42.0 (C(O)<u>C</u>H$_3$), 55.1 (O<u>C</u>H$_3$), 110.9 (<u>C</u>H), 119.9 (<u>C</u>H), 127.2 (<u>C</u>H), 128.4 (<u>C</u>H), 128.9 (<u>C</u>), 129.2 (<u>C</u>), 195.4 (<u>C</u>=O); *m/z* (EI) M^+ 210 (4), 135 (100).

m-Methoxy phenethyl thiol. [92]



130 **139**

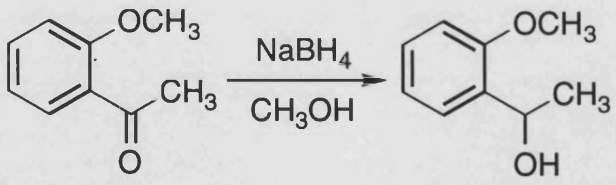
m-Methoxy phenethyl thioacetate (0.25 g, 1 mmol) in diethyl ether (3 ml) was treated with $LiAlH_4$ (1M soln. in Et_2O, 1.1 eq, 1.2 mmol, 1.2 ml) at 0 °C with stirring under nitrogen for 1 hour. The reaction was allowed to warm to room temperature and was stirred for an additional 1 hour.

The reaction mixture was re-cooled to 0 °C and HCl (0.1M) was added until the white precipitate disappears. The solution was diluted with diethyl ether (5 ml), washed with water (10 ml) and the organic layer collected. This was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The product thiol did not require any further purification.

Colourless oil (95%).

$\vartheta_{max.}$ / cm^{-1} 2979; δ_H (250 MHz, $CDCl_3$) 1.64 (3H, d, J=7.2, C<u>H</u>$_3$), 2.12 (1H, d, J=5.1, S<u>H</u>), 3.82 (3H, s, OC<u>H</u>$_3$), 4.62 (1H, dq, J=5.3, J=7.2, C<u>H</u>), 7.21 (5H, m, Ar<u>H</u>).

o-Methoxy phenethyl alcohol. [94]



132 **135**

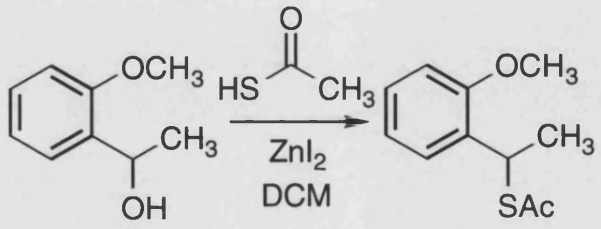
o-Methoxy acetophenone (5 g, 33.3 mmol) was dissolved in CH_3OH (70 ml) and $NaBH_4$ (1.1 eq, 36.6 mmol, 1.4 g) was added slowly with stirring at 0 °C until all starting material was consumed.

The reaction was extracted into diethyl ether (40 ml) and washed with water (40 ml). The organic layer was collected, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield the product alcohol.

Colourless oil (94%).

$\vartheta_{max.}$ / $^{cm-1}$ 3649; δ_H (250 MHz, $CDCl_3$) 1.45 (3H, d, J=5.4, C<u>H</u>$_3$), 3.76 (3H, s, OC<u>H</u>$_3$), 4.85 (1H, q, J=5.3, C<u>H</u>), 7.29 (5H, m, Ar<u>H</u>)

o-Methoxy phenethyl thioacetate. [91]



135 **129**

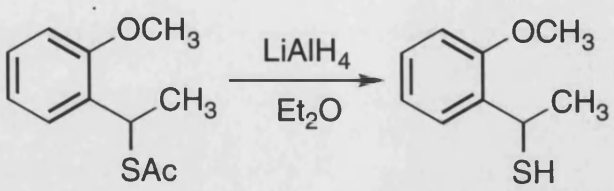
o-Methoxy phenethyl alcohol (3.9 g, 25.7 mmol) in dichloromethane (60 ml) was stirred with ZnI_2 (0.5 eq, 13 mmol, 4.1 g) and treated with thiolacetic acid (1.1 eq, 28.2 mmol, 2.14 g, 2.3 ml) with stirring at room temperature for 5 hours.

The reaction mixture was washed with water (70 ml), and the organic layer collected, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The product was purified using flash chromatography (10 % diethyl ether / petroleum ether).

Pale yellow clear oil (63%).

$\vartheta_{max.}$ / $^{cm-1}$ 1691; δ_H (400 MHz, $CDCl_3$) 1.65 (3H, d, J=7.3, C<u>H</u>$_3$), 2.26 (3H, s, C(O)C<u>H</u>$_3$), 3.78 (3H, s, OC<u>H</u>$_3$), 4.73 (1H, q, J=7.3, C<u>H</u>), 7.14 (5H, m, Ar<u>H</u>); δ_C (100 MHz, $CDCl_3$) 21.3 (<u>C</u>H$_3$), 30.2 (<u>C</u>H), 42.2 (C(O)<u>C</u>H$_3$), 55.4 (O<u>C</u>H$_3$), 116.5 (<u>C</u>H), 120.3 (<u>C</u>H), 127.5 (<u>C</u>H), 127.8 (<u>C</u>H), 128.2 (<u>C</u>), 129.4 (<u>C</u>), 195.5 (<u>C</u>=O); *m/z* (EI) M^+ 210 (21), 135 (100).

o-Methoxy phenethyl thiol. [92]



129 **138**

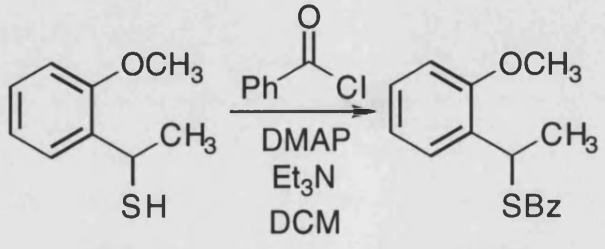
o-Methoxy thioacetate (0.3 g, 1.3 mmol) in diethyl ether (3 ml) was treated with $LiAlH_4$ (1M soln. in Et_2O, 1.1 eq, 1.47 mmol, 1.5 ml) with stirring under nitrogen at 0 °C for 1 hour. The solution was then allowed to warm to room temperature and the reaction was stirred for an additional hour.

The reaction mixture was re-cooled to 0 °C and HCl (0.1M) was added until the white precipitate disappears. The solution was diluted with diethyl ether (5 ml), washed with water (10 ml) and the organic layer collected. This was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The product thiol did not require any further purification.

Colourless oil (98%).

$\vartheta_{max.}$ / cm^{-1} 2989; δ_H (250 MHz, $CDCl_3$) 1.63 (3H, d, J=7.2, C<u>H</u>$_3$), 2.20 (1H, d, J=5.2, S<u>H</u>), 3.83 (3H, s, OC<u>H</u>$_3$), 4.64 (1H, dq, J=5.3, 7.2, C<u>H</u>), 7.23 (5H, m, Ar<u>H</u>); δ_C (68 MHz, $CDCl_3$) 24.2 (<u>C</u>H$_3$), 32.7 (<u>C</u>H), 56.4 (<u>C</u>H$_3$), 110.2 (<u>C</u>H), 121.6 (<u>C</u>H), 126.8 (<u>C</u>H), 130.1 (<u>C</u>H), 132.6 (<u>C</u>), 156 (<u>C</u>).

o-Methoxy phenethyl thiobenzoate. [91]



138 **140**

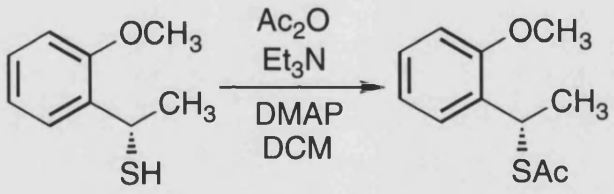
o-Methoxy phenethyl thiol (3.7 g, 20 mmol) in dichloromethane (40 ml) and triethylamine (1.1 eq, 22 mmol, 2.2 g) was stirred with 3-4 crystals of DMAP in a water bath under nitrogen. To this benzoyl chloride (1.1 eq, 22 mmol, 3.1 g) was added dropwise and the reaction mixture was stirred for 1 hour.

The solution was washed with water (30 ml) and the organic layer collected. This was dried ($MgSO_4$), filtered and concentrated *in vacuo.* The thiobenzoate was purified by flash chromatography (10% diethyl ether / petroleum ether).

Colourless oil (62%).

$\vartheta_{max.}$ / cm^{-1} 1686; δ_H (400 MHz, $CDCl_3$) 1.84 (3H, d, J=7.2, $C\underline{H}_3$), 3.96 (3H, s, $OC\underline{H}_3$), 5.32 (1H, q, J=7.3, C<u>H</u>), 7.57 (9H, m, Ar<u>H</u>); δ_C (68 MHz, $CDCl_3$) 18.4 ($\underline{C}H_3$), 38.3 (<u>C</u>H), 55.7 (O<u>C</u>H), 110.2 (<u>C</u>H), 111.1 (<u>C</u>H), 113.5 (<u>C</u>H), 127.0 (<u>C</u>H), 127.2 (<u>C</u>H), 127.4(<u>C</u>H), 128.5 (<u>C</u>H), 129.7 (<u>C</u>), 130.7 (<u>C</u>), 133.4 (<u>C</u>), 192.4 (<u>C</u>=O).

Derivatisation of o-methoxy phenethyl thiol.

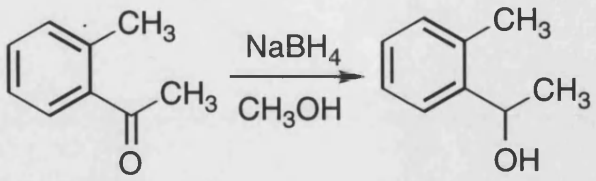


138 **129**

o-Methoxy phenethyl thiol (0.1 g, 0.5 mmol) in dichloromethane (1 ml), triethylamine (1.1 eq, 0.57 mmol, 0.066 g), acetic anhydride (1.1 eq, 0.57 mmol, 0.058 g) and 1 crystal of DMAP was stirred at room temperature for 1 hour.

The reaction mixture was extracted into diethyl ether (10 ml) and washed with water (15 ml). The organic layer was collected, dried ($MgSO_4$), filtered, and concentrated *in vacuo* and the product was purified using prep. TLC (5% diethyl ether / petroleum ether).

o-Methyl phenethyl alcohol. [94]



134 **137**

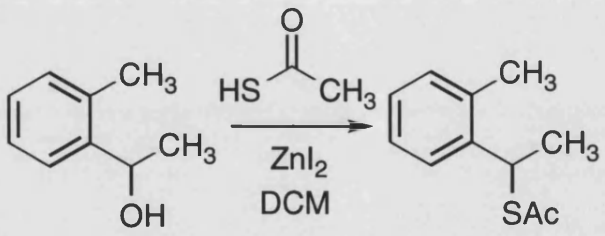
Sodium borohydride (1.1 eq, 32 mmol, 1.28 g), was slowly added with stirring to o-methyl acetophenone (4 g, 30 mmol) dissolved in CH_3OH (50 ml) at 0 °C. After the addition, the reaction was allowed to warm to room temperature and stirred until all starting material was consumed.

The reaction mixture was extracted into diethyl ether (30 ml) and washed with water (50 ml). The organic layer was collected, dried ($MgSO_4$), filtered and concentrated *in vacuo*. No further purification was required.

Opaque oil (94%).

$\vartheta_{max.}$ / cm^{-1} 2701; δ_H (250 MHz, $CDCl_3$) 1.61 (3H, d, J=7.3, C<u>H</u>$_3$), 2.28 (3H, s, C(O)C<u>H</u>$_3$), 2.32 (3H, s, ArC<u>H</u>$_3$), 4.71 (1H, q, J=7.2, C<u>H</u>), 7.18 - 7.21 (5H, m, Ar<u>H</u>).

o-Methyl phenethyl thioacetate. [91]



137 **131**

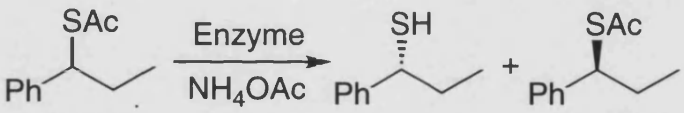
o-Methyl phenethyl alcohol (2.9 g, 21.3 mmol) in dichloromethane (45 ml) was stirred with ZnI_2 (0.5 eq, 11 mmol, 3.4 g) and treated with thiolacetic acid (1.1 eq, 23.5 mmol, 1.78 g, 1.9 ml) with stirring at room temperature. The solution was stirred for 6 hours.

The reaction mixture was washed with water (40 ml) and the organic layer collected and filtered through a plug of celite. The solvent was removed *in vacuo* and the product was purified by flash chromatography (30% diethyl ether / petroleum ether).

Colourless oil (81%).

$\vartheta_{max.}$ / $^{cm-1}$ 1703; δ_H (250 MHz, $CDCl_3$) 1.61 (3H, d, J=7.1, $CHC\underline{H}_3$), 2.29 (3H, s, $ArC\underline{H}_3$), 2.40 (3H, s, $C(O)C\underline{H}_3$), 4.89 (1H, q, J=7.2, C<u>H</u>), 7.20 - 7.23 (5H, m, Ar<u>H</u>); δ_C (100 MHz, $CDCl_3$) 19.7 ($\underline{C}H_3$), 20.2 ($\underline{C}H_3$), 39.5 (<u>C</u>H), 55.4 ($C(O)\underline{C}H_3$), 111.5 (<u>C</u>H), 119.3 (<u>C</u>H), 126.9 (<u>C</u>H), 127.4 (<u>C</u>H), 127.7 - 129.4 (<u>Ar-C</u>), 195.5 (<u>C</u>=O); *m/z* (EI) M^+ 194 (46).

Enzymatic hydrolysis of 1-phenyl-1-thioacetoxy propane.

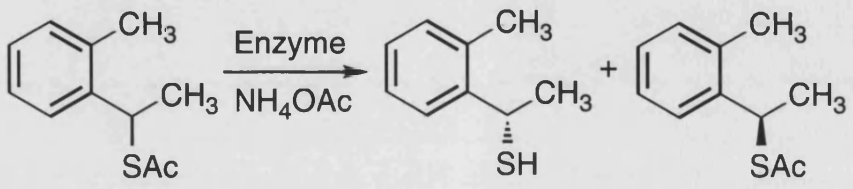


128 **145** **128**

1-Phenyl-1-thioacetoxy propane (150 µl, 0.8 mmol) in ammonium acetate buffer (0.1M, 1 ml) was treated with the enzyme (20 mg) and stirred at 25 °C.

The reaction products were extracted into diethyl ether, filtered through a plug of silica and the solvent was removed *in vacuo*.

Enzymatic hydrolysis of o-methyl phenethyl thioacetate.

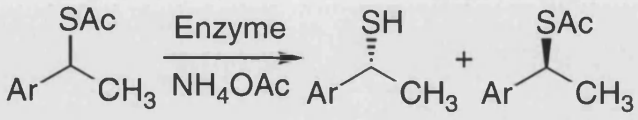


131 **146** **131**

o-Methyl phenethyl thioacetate (100 μl, 0.5 mmol) in ammonium acetate buffer (0.1M, 2 ml) was stirred with the enzyme (15 mg) at 22 °C.

The reaction products were extracted into diethyl ether (2 ml) and filtered through a plug of silica. The solvent was stripped *in vacuo*.

Enzymatic hydrolysis of m-methoxy phenethyl thioacetate.



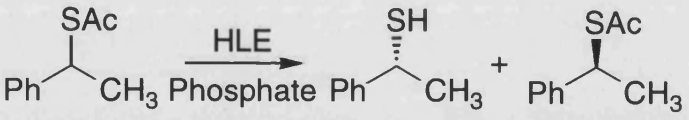
130 **139** **130**

Ar = m-$(OCH_3)C_6H_4$

m-Methoxy phenethyl thioacetate (100 μl, 0.5 mmol) in ammonium acetate buffer (0.1M, 1 ml) and the enzyme (20 mg) was stirred at 22 °C.

The reaction mixture was extracted into diethyl ether (1 ml) and filtered through a plug of silica. The solvent was removed *in vacuo*.

Enzymatic hydrolysis of phenethyl thioacetate.

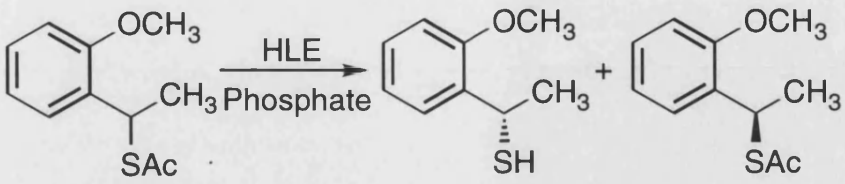


127 **120** **127**

Phenethyl thioacetate (0.3 g, 1.7 mmol) in phosphate buffer (0.1M, 1.5 ml) was treated with HLE (60 mg) and stirred at 50 °C for 7 days.

The reaction mixture was extracted into dichloromethane (3 ml) and washed with water (8 ml). The organic layer was collected, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Reaction products were separated using prep. TLC (10% diethyl ether / petroleum ether).

Enzymatic hydrolysis o-methoxy phenethyl thioacetate.

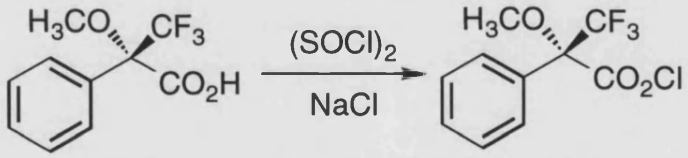


129 **138** **129**

o-Methoxy phenethyl thioacetate (0.5 g, 2.2 mmol) in the phosphate buffer (0.1M, 2 ml) was stirred with HLE (150 mg) at 23 °C for 7 days.

The solution was extracted with diethyl ether (10 ml) and washed with water (15 ml). The organic layer was collected, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Reaction products were separated and purified using prep. TLC (5% diethyl ether / petroleum ether).

(S)-α-Methoxy-α-triflouromethyl phenylacetyl chloride (MTPACl). [95]



142 **143**

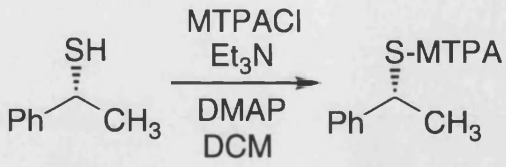
(R)-α-Methoxy-α-triflouromethyl phenylacetic acid (R)-(MTPA) (0.5 g, 2 mmol) in thionyl chloride (5 eq, 10 mmol, 1.27 g, 2.1 ml) was stirred with NaCl (0.05 eq, 0.006 g) at reflux for 2 days under nitrogen.

The thionyl chloride was removed *in vacuo* and the product acid chloride purified using flash chromatography (20% diethyl ether / petroleum ether).

Colourless oil (85%).

B.pt. 55.8 °C.

Moshers ester of phenethyl thiol. [93]



120 **144**

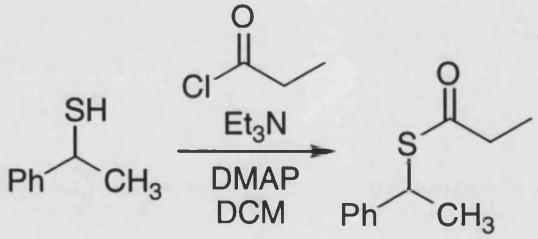
Phenethyl thiol (3 mg, 0.02 mmol) in dichloromethane (0.5 ml), and triethylamine (1.1 eq, 0.02 mmol, 2.2 mg), was treated with 1 crystal of DMAP and MTPACl (1.1 eq, 0.02 mmol, 5 mg). The reaction solution was stirred in a water bath over 2 hours under nitrogen.

The reaction products were extracted into dichloromethane (1 ml) and washed with water (2 ml). The organic layer was collected, dried ($MgSO_4$), filtered and concentrated in vacuo. The product was purified using prep. TLC (5% diethyl ether / petroleum ether) yielding the moshers ester.

Colourless oil (87%).

δ_H (400 MHz, $CDCl_3$) 1.68 (3H, d, J=7.20, $CHC\underline{H}_3$-major), 1.62 (3H, d, J=7.23, $CHC\underline{H}_3$-minor).

Phenethyl thioethanoate. [90]



120 **147**

Phenethyl thiol (0.3 g, 2 mmol) in dichloromethane (5 ml) and triethylamine (1.1 eq, 2.4 mmol, 0.22 g) was treated dropwise with propanoyl chloride (1.1 eq, 2.4 mmol, 0.24 ml). The reaction mixture was stirred at room temperature for 1 hour.

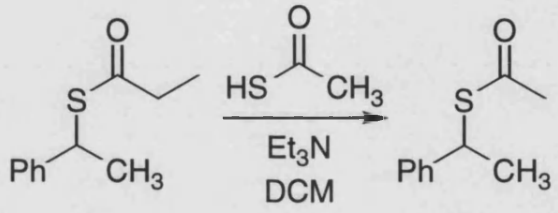
The solution was washed with water (10 ml) and diluted with dichloromethane (10 ml). The organic layer was collected, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The product was

purified using flash chromatography (5% diethyl ether / petroleum ether).

Pale yellow clear oil (74%).

$\vartheta_{max.}$ / cm^{-1} 1695; δ_H (250 MHz, $CDCl_3$) 1.15 (3H, t, J=6.95, $CH_2C\underline{H}_3$), 1.65 (2H, d, J=6.61, $CHC\underline{H}_3$), 2.54 (2H, q, J=6.95, $C\underline{H}_2$), 4.75 (1H, q, J=6.61, C<u>H</u>), 7.21 - 7.27 (5H, m, Ar<u>H</u>); δ_C (68 MHz, $CDCl_3$) 9.5 ($\underline{C}H_3$), 22.3 ($\underline{C}H_3$), 37.2 ($\underline{C}H_2$), 42.6 (<u>C</u>H), 112.5 (<u>C</u>H), 127.1 - 128.5 (<u>Ar-C</u>), 193.3 (<u>C</u>=O).

Substitution of phenethyl thioethanoate.



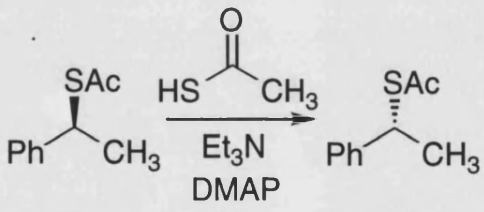
147 **127**

Phenethyl thioethanoate (0.5 eq, 2.6 mmol) in dichloromethane (5 ml), thiolacetic acid (5 eq, 13 mmol, 1.04 ml), and triethylamine (5 eq, 12.8 mmol, 1.3 g) was stirred at 23 °C over 18 hours.

The reaction mixture was washed with water (10 ml) and diluted with dichloromethane (10 ml). The organic layer was collected, dried ($MgSO_4$), filtered, and concentrated *in vacuo*. Flash chromatography (5% diethyl ether / petroleum ether) of the reaction products yielded phenethyl thioacetate.

Colourless oil (79%).

Racemisation phenethyl thioacetate.



127 **127**

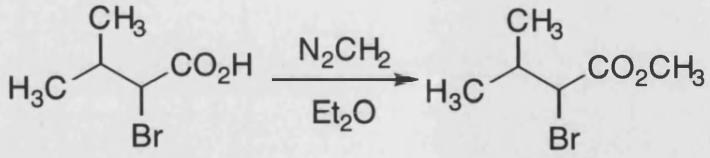
(S)-Phenethyl thioacetate (0.1 g, 0.g mmol) in dichloromethane (1 ml), triethylamine (5 eq, 2.8 mmol, 0.28 g), and thiolacetic acid (5 eq, 2.6 mmol, 0.21 ml) was stirred at 40 ^{o}C for 7 days.

The solution was washed with water (3 ml) and the organic layer collected. This was dried ($MgSO_4$), filtered, and concentrated *in vacuo*. The racemic product was purified using prep. TLC (5% diethyl ether / petroleum ether).

Colourless oil (34%).

6.5 Chapter 5 Experimental.

Methyl-2-bromo-3-methyl butanoate. [96]



174 **173**

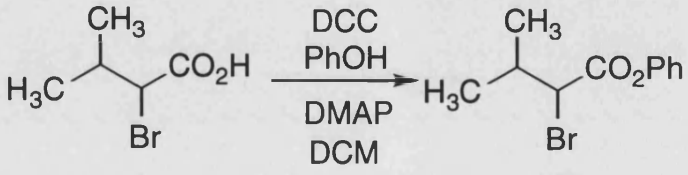
2-Bromo-3-methyl butanoic acid (0.5 g, 27 mmol) was dissolved in diethyl ether (30 ml) and stirred at room temperature under N_2. Diazald (2.5 eq, 1.5 g) in ethanol (50 ml) was placed under a nitrogen atmosphere and 3-4 pellets of KOH were added. The diazomethane produced was bubbled through the acid solution until all diazomethane was consumed.

The solvent was removed *in vacuo* to yield methyl-2-bromo-3-methyl butanoate with no further purification required.

Colourless oil (98%).

ϑ_{max} / $^{cm-1}$ 1742; δ_H (400 MHz, $CDCl_3$) 1.16 (3H, d, J=6.6, $(CH_3)CHC\underline{H}_3$), 1.19 (3H, d, J=6.6, $C\underline{H}_3CH(CH_3)$), 2.33 (1H, m, J=6.6, J=6.7, $(CH_3)C\underline{H}CH_3$), 3.80 (3H, s, $C(O)OC\underline{H}_3$), 4.18 (1H, d, J=6.7, $C\underline{H}(Br)$); δ_C (100 MHz, $CDCl_3$) 24.3 ($\underline{C}H_3$), 24.5 ($\underline{C}H_3$), 52.7 ($\underline{C}H$), 53.0 ($\underline{C}H$), 61.2 ($O\underline{C}H_3$), 169.1 ($\underline{C}$=O).

Phenyl-2-bromo-3-methyl butanoate. [97]



174 **175**

2-Bromo-3-methyl butanoic acid (1 g, 5.5 mmol) was dissolved in dichloromethane (20 ml), phenol (1 eq, 0.51 g) and 1-2 crystals of

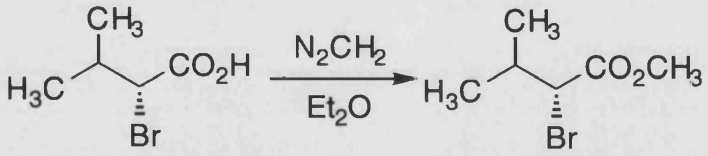
DMAP was added at 0 °C. DCC (1.1 eq, 6 mmol, 1.6 g) was added and the reaction mixture was stirred at room temperature overnight.

The solution was repeatedly washed through a plug of silica with diethyl ether (4 x 20 ml) and petroleum ether (4 x 20 ml) and concentrated *in vacuo*. The product was purified using flash chromatography (10% diethyl ether / petroleum ether) to yield the phenolic ester.

Yellow clear oil (74%).

ϑ_{max} / cm^{-1} 1711; δ_H (400 Mhz, $CDCl_3$) 1.15 (3H, d, J=6.5, $C\underline{H}_3CH(CH_3)$), 1.19 (3H, d, J=6.5, ($CH_3CH(C\underline{H}_3)$, 2.37 (1H, dq, J=6.5, J=6.7, $CH_3C\underline{H}(CH_3)$), 4.23 (1H, d, J=6.7, $CHC\underline{H}(Br)$), 7.10-7.12 (2H, m, Ar<u>H</u>), 7.21-7.27 (1H, m, Ar<u>H</u>), 7.35-7.41 (2H, m, Ar<u>H</u>); δ_C (100 MHz, $CDCl_3$) 19.9 ($\underline{C}H_3$), 32.4 ($\underline{C}H_3$), 54.1 (<u>C</u>H), 55.7 (<u>C</u>H), 121.3 (2x<u>C</u>H), 126.4 (2x<u>C</u>H), 129.8 (<u>C</u>H), 150.4 (<u>C</u>), 167.9 (<u>C</u>=O).

Derivatisation of 2-Bromo-3-methyl butanoic acid.

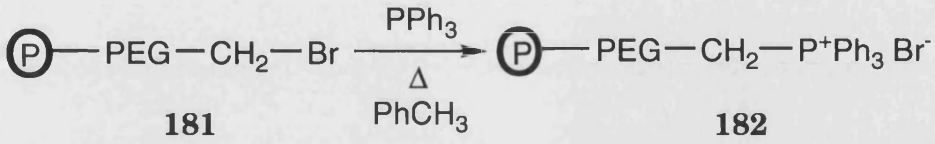


174 **173**

2-Bromo-3-methyl butanoic acid (15 mg, 0.08 mmol) was dissolved in diethyl ether (5 ml) and stirred at 0 °C. Diazald (1.5 eq, 28 mg) was dissolved in ethanol (10 ml) and treated with 1-2 pellets of KOH in a seperate flask. The diazomethane was bubbled through the acid solution at 0 °C until all diazomethane was consumed.

Solvent was stripped *in vacuo* to yield methyl-2-bromo-3-methyl butanoate with no further purification required.

Tentagel-Triphenylphosphonium bromide.

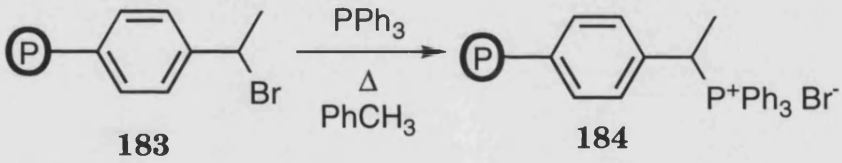


181 **182**

Brominated TG resin (0.3 g, 0.1 mmol loading of bromide) in toluene (15 ml) and triphenylphosphine (7 eq, 0.66 mmol, 0.17 g) was refluxed for 6 hours.

The reaction was cooled to room temperature and washed consecutively with dichloromethane (5 x 30 ml) and methanol (4 x 30 ml). The remaining solid was collected and dried overnight in a vacuum oven at 50 °C.

Wang triphenylphosphonium bromide.

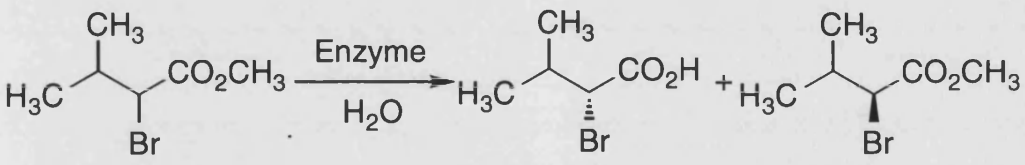


183 **184**

Brominated Wang resin (0.2 g, 0.2 mmol loading of bromide) in toluene (30 ml) and triphenylphosphine (7 eq, 0.36 g) was refluxed for 4 hours.

The reaction was allowed to cool to room temperature and washed repeatedly with dichloromethane (5 x 30 ml) and methanol (4 x 30 ml). The solid was collected and dried over night in a vacuum oven at 50 °C.

Enzymatic hydrolysis of methyl-2-bromo-3-methyl butanoate.



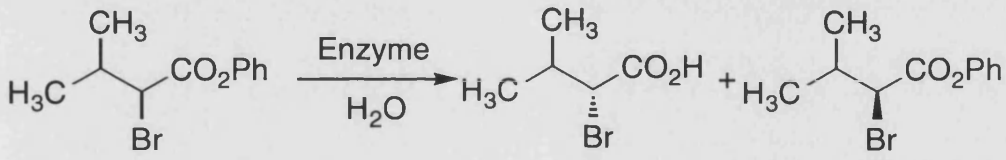
173 **174** **173**

Methyl-2-bromo-3-methyl butanoate (0.1 g, 0. 5 mmol) was stirred in water (20 ml) with the enzyme (20 mg). The reaction was maintained at 30 °C over 18 hours in a pH stat. equipment. The pH was maintained at 7.2 via continual addition of 0.1M KOH.

The reaction was extracted with diethyl ether (10 ml), separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield methyl-2-bromo-3-methyl butanoate.

The aqueous layer was acidified to ~ pH 1.5 with 1M HCl, extracted into diethyl ether (10 ml), separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield 2-bromo-3-methyl butanoic acid.

Enzymatic hydrolysis of Phenyl-2-bromo-3-methyl butanoate.



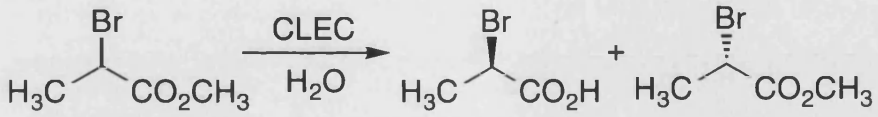
175 **174** **175**

Phenyl-2-bromo-3-methyl butanoate (0.26 g, 1 mmol) was stirred in water (25 ml) and enzyme (30 mg)was added. The reaction solution was stirred at 30 °C for 48 hours in a pH stat. apparatus maintaing the pH at 7.0 by the continual addition of 0.1M KOH.

The reaction mixture was extracted with diethyl ether (10 ml), separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield phenyl-2-bromo-3-methyl butanoate.

The aqueous layer was acidified to ~ pH 1.5 with 0.1M HCl, extracted into diethyl ether (10 ml), separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield 2-bromo-3-methyl butanoic acid.

Enzymatic hydrolysis of methyl-α-bromo propionate.



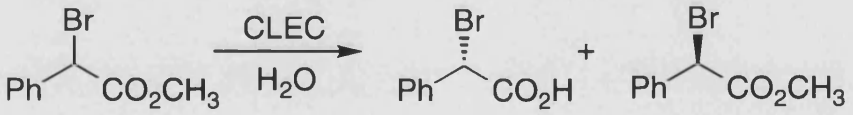
169 **170** **169**

Methyl-α-bromo propionate (0.17 g, 1 mmol) in water (25 ml) was treated with CLEC enzyme (5 mg) and stirred at room temperature whilst maintaining pH at 7.5 via continual addition of 0.1M KOH in a pH stat. apparatus.

The reaction was extracted into diethyl ether (10 ml), separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield methyl-α-bromo propionate.

The aqueous layer was acidified to ~ pH 1.5 with 0.1M HCl and extracted with diethyl ether (10 ml), separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield α-bromo propionic acid.

Enzymatic hydrolysis of methyl-α-bromo phenyl acetate.



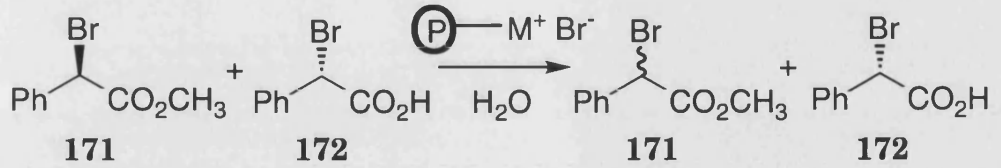
171 **172** **171**

Methyl-α-bromo phenyl acetate (0.25 g, 1 mmol) in water (30 ml) was treated with the CLEC enzyme (5 mg) at room temperature. The reaction was maintained at pH 7.0 by the continual addition of 0.1M KOH via an auto-titrator.

The reaction solution was extracted into diethyl ether (15 ml), separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield methyl-α-bromo phenyl acetate.

The aqueous layer was acidified to ~ pH 1.5 with 0.1M HCl and extracted with diethyl ether (15 ml), separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield α-bromo phenyl acetic acid.

Methyl ester selective racemisation of methyl-α-bromo phenyl acetate.

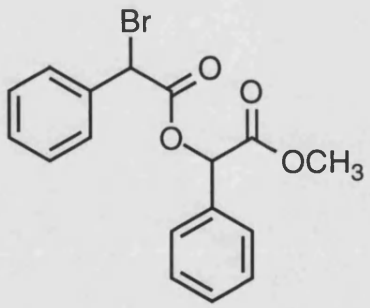


171 **172** **171** **172**

Methyl-α-bromo phenyl acetate and α-bromo phenyl acetic acid of known mass and enantiomeric excess in water (30 ml) was treated with the immobilised phosphonium bromide salt (0.4 eq of bromide) and stirred at room temperature for 2 hours.

The resin was collected by filtration and the remaining aqueous solution acidified to ~ pH 1.5 with 0.1M HCl, extracted with diethyl ether (10 ml), separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield the reaction products.

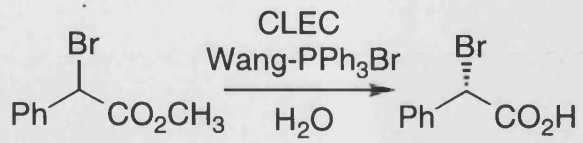
Methyl phenyl acetate-α-bromo phenyl acetate.



178

ϑ_{max} / $^{cm-1}$ 1732; δ_H (400 Mhz, $CDCl_3$) 3.73 (3H, s, $OC\underline{H}_3$), 5.51 (1H, s, C<u>H</u>), 5.98 (1H, s, C<u>H</u>), 7.21 -7.61 (10H, m, Ar<u>H</u>); δ_C (100 MHz, $CDCl_3$) 57.6 ($O\underline{C}H_3$), 79.2 (<u>C</u>H), 80.1 (<u>C</u>H), 127.0 (2x<u>C</u>H), 127.3 (2x<u>C</u>H), 129.1 (2x<u>C</u>H), 131.7 (2x<u>C</u>H), 131.9 (2x<u>C</u>H), 132.4 (<u>C</u>), 136.3 (<u>C</u>), 185.2 (<u>C</u>=O), 189.4 (<u>C</u>=O); *m / z* (EI) 362 (28, M^+), 213 (54); (Found: M^+, 362.0162. C, 56.4; H, 4.2; Br, 21.8; O, 17.7); (C17H15BrO4 requires *M*, 362.0154. C, 56.3; H, 4.2; Br, 21.8; O, 17.6).

Dynamic resolution of methyl-α-bromo phenyl acetate.



171 **172**

Methyl-α-bromo phenyl acetate (0.12 g, 0.5 mmol) in water (30 ml) was emulsified in an auto titrator. Wang-PPh_3Br (0.46 g, 0.4 mmol) was added along with CLEC-CR (10 mg) and maintained at pH 7.0 by the continual addition of 0.1M KOH. The reaction was stopped when no more base was added.

The polymer bound phosphonium salt and CLEC-CR were filtered from the reaction and the aqueous solution acidified to ~ pH 1.5 with 0.1M HCl. Reaction was extracted with diethyl ether (20 ml), separated, dried ($MgSO_4$), filtered, and concentrated *in vacuo* to yield α-bromo phenyl acetic acid.

7.0 List of Relevant Publications

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